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SEA BUCKTHORN CULTIVAR AND ORCHARD DEVELOPMENT IN SASKATCHEWAN

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Sea buckthorn cultivar and orchard development in Saskatchewan

ADF Project No. 98000273

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A. Abstract

Sea buckthorn (*Hippophae rhamnoides* L.) is a winter hardy woody plant, which was introduced to the Canadian prairies from Siberia as a shelterbelt species in the 1930s. The species has adapted well to Saskatchewan conditions.

Since 1995, the PFRA Shelterbelt Centre, Indian Head and the Department of Plant Sciences, University of Saskatchewan have been collaborating on a research project to develop sea buckthorn for cultivation and processing in Saskatchewan (SADF project #95000439-19BR). This project focused on orchard management technology, establishment of a base population and selection of superior genotypes for commercial cultivation in Saskatchewan. The major achievements of the project are (a) the establishment of a breeding base of 2000 genetically diverse accessions of sea buckthorn from around the world, (b) selection of over 200 improved lines, (c) development of vegetative propagation techniques, (d) determination of quality and chemical composition of the fruit and seeds, (e) establishment of a 20 acre agroforestry intercropping demonstration, and (f) development of orchard technology, such as spacing, harvesting techniques, optimum harvest dates.

Our work has also shown that sea buckthorn grown in Saskatchewan has a higher oil content than fruit grown anywhere else in the world. Chemical analysis has shown that fruit grown in Saskatchewan is of very high quality, rich in vitamins A, B_1, B_2, C, D, tocopherols, carbohydrates, 30% protein, organic acids, amino acids and 20 mineral elements.

Flavonoid content and profile in leaves and berries of four species (*H. rhamnoides*, *H. gyantensis*, *H. neurocarpa*, H. *mongolica)* of different origin and four subspecies of *H. rhamnoides: rhamnoides, sinensis , turkestanica* and *mongolica (*cultivar Indian Summer) was investigated from 1999 to 2001. Flavonoid content, expressed as isorhamnetin equivalents, ranged from 0.9% to 2.0% in air-dried leaves, and 0.18% to 0.56% in lyophilized berry flesh. Leaves and berries of recommended variety RC-4 contained the highest levels of flavonoids in 1999 and 2000. Flavonoid composition in sea buckthorn is complex; leaves consists of at least 13 and berries of at least 19 distinct compounds. Difference in flavonoid composition was revealed at the species and subspecies level. Four pure flavonol glycosides were isolated from sea buckthorn leaves, two with quercetin aglycone and two with isorhamnetin aglycone.

Sea buckthorn has attracted a great deal of interest in Canada. Processors have been searching for increasing volumes of sea buckthorn fruit. It is estimated that processing plants will require 20 million kilograms of sea buckthorn fruit annually, translating into 4,000 acres of sea buckthorn orchard production. The absence of selected and tested cultivars was a major constraint limiting orchard production and fruit processing. The SADF supported project has alleviated this limitation. The project has identified four promising genotypes of sea buckthorn, which can be grown commercially in Saskatchewan, giving our industry a competitive advantage.

B. Executive Summary

Project Background

Sea buckthorn accessions were introduced to the Canadian prairies from Siberia as a shelterbelt species in the 1930s. Sea buckthorn (*Hippophae rhamnoides* L.) is winter hardy, and has adapted well to Saskatchewan conditions Climatic and soil conditions in Saskatchewan are ideally suited for high quality fruit production. It is a fascinating nutraceutical plant. The nutritional and medicinal value of sea buckthorn has been exploited in Eurasia for years. Chemical analysis has shown that fruit grown in Saskatchewan is of extremely high quality, with above average oil content, rich in vitamins A, B_1, B_2, C, D, tocopherols, carbohydrates, 30% protein, organic acids, amino acids and 20 mineral elements. Our work has also shown that sea buckthorn grown in Saskatchewan potentially has a higher oil content than fruit grown anywhere else in the world, giving our industry a competitive advantage.

Sea buckthorn has attracted a great deal of interest in Canada. Processors are searching for increasing volumes of sea buckthorn fruit. Industry estimates are that processing plants will require 20 million kilograms of sea buckthorn fruit annually, translating into 4,000 acres of sea buckthorn orchard production. The absence of selected and tested cultivars was a major constraint limiting orchard production and fruit processing.

Since 1995, the PFRA Shelterbelt Centre and Department of Plant Sciences, University of Saskatchewan have been collaborating on a research project on Development and Demonstration of Sea Buckthorn for Cultivation and Processing in Saskatchewan (SADF project #95000439-19BR). This project focused on orchard management technology, establishment of a base population and selection of superior genotypes for commercial cultivation in Saskatchewan. The major results of this project were the establishment of a breeding base of 2000 genetically diverse accessions of sea buckthorn from around the world, selection of over 200 improved lines, development of vegetative propagation techniques, determination of quality and chemical composition of the fruit and seeds, establishment of a 20 acre agroforestry intercropping demonstration and development of orchard technology such as spacing, harvesting techniques, optimum harvest dates.

Currently, companies are dependent on sourcing sea buckthorn fruit from shelterbelts and wildlife plantings grown from seed or orchards established from seed. Because sea buckthorn is a dioecious species, these plantings are a mixture of male and female plants and mechanical harvesting is impossible. The absence of sea buckthorn cultivars that can be harvested mechanically is major impediment to the further development of a sea buckthorn industry in Saskatchewan. The development of a mechanical harvester is currently under way in a joint SADF and Manitoba Rural Adaptation funded project at PAMI in Portage La Prairie, MB. The production of new cultivars would increase the viability and productivity of Saskatchewan fruit growers and

processors. New cultivar development will allow growers to provide a consistent and reliable supply of high quality fruit, which is not available to processors at this time.

Project Objectives and Activities

The objectives of this project are to expand and support the development of sea buckthorn in Saskatchewan. This will positively impact the fruit, nursery and processing industries. The major focus of the project was to build on previously SDAF funded work related to breeding and cultivar development and orchard technology.

1. Cultivar Selection and Development

Female and male cultivars selected between 1996 and 1999 were intensively evaluated. This involved propagating selections and detailed evaluation of fruit and seed characteristics. Release of the top five female cultivars 6582, RC-4, OL-6, FR-14 and 6476 was initiated the 2001. Outstanding characteristics of these cultivars include: absence of thorns (6582 and 6476), high fruit yield (6582, OL-6) easily harvested (6582, 6476, FR-14), exceptional nutritional profile (RC-4). One male clone (H6-121) with superior pollen production has been released. These cultivars are being propagated by commercial nurseries for sale to sea buckthorn growers.

2. Germplasm Nursery

A sea buckthorn germplasm nursery was established at the PFRA Shelterbelt Centre. About 2000 accessions of four species and 5 subspecies of *Hippopha* are being grown in this nursery. Individual plants with desirable characteristics were selected from this nursery for hybridization. Specific traits of interest are dwarf growth form, thornlessness, degree of suckering, stem form and fruiting characteristics. Diversity of populations was studied in depth. Hybridization was conducted by harvesting pollen form male plants of diverse origin and characteristics and pollinating selected female individuals with desirable traits. The resulting seeds were propagated and will be planted in a progeny test in 2002 for advanced generation selection.

3. Orchard Management

The project developed and demonstrated pruning techniques that facilitate mechanical harvest using tree and branch shakers. The open centre pruning system aimed at developing a three branch system was demonstrated at two experimental orchards. The optimum male:female ratio of 1:6 to maximize fruit yield was demonstrated in an orchard established using clonal plant material. Various harvesting techniques were evaluated. It was determined that shaking was the most effective harvesting method. The project collaborated with the PAMI harvester study by providing test orchards for harvester evaluation. Project staff also assisted in harvester evaluation.

4. Propagation

Seed and softwood cutting propagation techniques for sea buckthorn were developed. This included studies to determine optimum seed treatment; timing of cutting collection, media selection and rooting hormones.

5. Technology Transfer

Technology transfer included direct grower support by attending workshops, field days, newsletter submissions and technical enquiries. During the project 760 individual enquiries related to sea buckthorn were answered, one fact sheet was printed and a sea buckthorn workshop was hosted.

6. Flavonoid Study

Sea buckthorn is a rich source of unsaturated fatty acids, vitamins C and E, carotenoids, phytosterols, and flavonoids. Whereas berries, seed, and leaves are marketed in the form of food (juice, tea), dermatological products (ointments and oils), and dietary supplements (extracts, concentrates) worldwide, very limited studies on natural compounds such as flavonoids from leaves have been conducted to date. This project addressed total flavonoid content and flavonoid profile in leaves and berries of four *Hippophae* species (*H. rhamnoides*, *H. gyantensis*, *H. neurocarpa*, H. *mongolica)* and four subspecies of *H. rhamnoides: rhamnoides, sinensis, turkestanica* and *mongolica (*variety Indian Summer). Samples were taken from plants of varied origin (China, Russia, Finland and Canada) grown in the sea buckthorn germplasm nursery established as a part of this project at the PFRA Shelterbelt Centre, Indian Head. Samples from twenty four selections of Indian Summer collected across Saskatchewan were also studied.

Three-year study of 166 leaf and 59 berry samples showed that flavonoid content, expressed as isorhamnetin equivalents, ranged from 0.92% to 2.00% for air-dried leaves, and 0.18% to 0.56% for lyophilized berry flesh. Selections of Indian Summer that contained high amount of flavonoids in leaves were RC-4 (1.7 to 2.0%), OL-6 (1.4-1.8%) and FR-13 (1.3 to 1.7%). Selection RC-4 contained 0.4-0.5% of flavonoids in lyophilized berry flesh over three years of this study. Single highest amount of flavonoids in berries (0.56%) was found for *H. rhamnoides* ssp. *sinensis* in 2001. Leaves of *H. rhamnoides* ssp. *rhamnoides* contained lower amount of flavonoids than all other sea buckthorn species and subspecies tested. On the dry weight basis, leaves are considerably richer source of flavonoids than berries. The investigation of the flavonoid profile by RP-HPLC revealed that berries and leaves contain at least 19 and 13 distinct compounds, respectively. Flavonoid composition varies at the species and subspecies level. Given that flavonoids exhibit a variety of activities beneficial to human health (e.g., adaptogenic, antinflammatory, antioxidative, antiviral, antitumor, and immunostimulatory), it is recommended that selection for high flavonoid content be considered in future genetic improvement studies of sea buckthorn. Flavonoids can be extracted effectively from sea buckthorn leaves by simple extraction with water at 30 °C, which opens opportunities for new product development from sea buckthorn leaves and berries at the cottage level to large scale.

Flavonoids were separated from a crude 30% methanol extract of dry R-C4 leaves using a RP-C18 chromatographic cartridge, and then further fractionated by LP-LC on a LiChroprep RP-18 column using gradient elution. Four pure flavonol glycosides, two with quercetin as the aglycone were isolated (namely quercetin 3,7-O-diglucoside, MW 626; quercetin 3-O-glucoside-7-O-rhamnoside, MW 61), and two with isorhamnetin as aglycone. The chemical structures of two quercetin glycosides were determined using negative-ion FAB-MS, ^{1}H-NMR,^{13}C-NMR, UV spectra, bathochromic shifts after addition of a diagnostic reagent, and by comparison of our data with literature information. The presence of quercetin as the aglycone was also confirmed by acid hydrolysis of isolated compounds followed by HPLC analysis of hydrolysis products using quercetin as a reference standard.

C. Technical Report

1.0. Cultivar Development and Genetic Survey

1.1. Cultivar Development and Release

Sea buckthorn can be propagated sexually or asexually. Sexual propagation is accomplished through seed production. Because of sea buckthorn's considerable genetic diversity, propagation by seed can yield progeny that are very diverse phenotypically and genotypically. Height, form, thorniness and fruit characteristics can vary significantly from the parent plants. Also, seedlings are a mixture of male and female plants. The sex of these seedlings is very difficult to discern until flowering, which begins 4 – 5 years after germination. For these reasons, sexual propagation is not a desirable method of propagating cultivars for orchard use.

Asexual propagation, or vegetative propagation, involves producing clones through cuttings, suckers or tissue culture. Clones are genetically identical to the parent plant, making this the ideal method for propagating cultivars. With this method, the desirable characteristics for which the cultivar was selected (high yield, compact form, etc.) can be carried forward to the next generation. Additionally, the sex of the vegetatively propagated plant is pre-determined, allowing for the ideal male: female ratio of 1: 6 to be achieved in an orchard.

1.1.1. Seedling Cultivars

A seed source very common to the Canadian prairies is cultivar Indian Summer. This seed source belongs to the subspecies *mongolica,* which was introduced to the prairies in the 1930's by Dr. L. Skinner at Dropmore, Manitoba. Dr. Skinner obtained the seeds from a colleague in the former Soviet Union. The Shelterbelt Centre obtained clones of Dr. Skinner's plants in 1963 and seeds were collected from these clones to be planted into hedges. These hedges have subsequently provided seeds for the Shelterbelt Centre's shelterbelt program, which has seen sea buckthorn seedlings being planted across the Canadian prairies.

1.1.2. Clone Selection

Sea buckthorn planted in Saskatchewan shelterbelts was surveyed and superior cultivars were selected from these populations. Selection criteria included fruit yield, fruit and seed characteristics, skin toughness, thorniness, growth habit and harvesting ease. The top selections were released to commercial growers for propagation and eventual sale to growers. A total of 50 sea buckthorn selections from 11 locations in Saskatchewan that had been identified during the previous project were chosen for evaluation. The objective was to characterize the fruit and seed characteristics of the selections and finalize the top five selections for grower release.

Fruit collection was conducted during the fall of 1999, 2000 and 2001 when fruit was ripe. Ripening varied between years and from site to site and between selections. Ripening began in late August and concluded in late September. Selection data is shown in Appendix Table 2. Generally, ripening was earlier in 2001 than 1999 or 2000. Fruit samples (100) were removed from each selection. Seeds from each selection were retained for determination of seed oil content. Fruit removal force, fruit weight (g/100 fruit), pedicel length and seed weight (g/100 seeds) was recorded for each selection. Harvesting index, which is an estimate of ease of harvesting by shaking, was determined for selection using the following formula:

$$\textit{Harvesting Index} = \frac{\textit{(fruit weight) x (pedicel length)}}{\textit{(removal force)}}$$

1.1.3. Chemical Profiles of Sea Buckthorn Selections

1.1.3.1. Fatty Acid Profiles of Sea Buckthorn Selections

Fatty acid composition was determined using Gas Chromatography (GC) for seeds from selected plants. The analysis was conducted by the Crop Utilization Section, Agriculture and Agri-Food Canada, Saskatoon. The fatty acid composition of the samples was characterized by high levels of unsaturated linoleic and linolenic acid and oleic acid. The range of fatty acid composition was palmitic 5.72 - 7.33 %, stearic 2.59 - 3.32 %, oleic 14.66 - 28.66 %, linoleic 31.5 - 39.6 %, linolenic 27.37 - 41.35 % and others 1.74 - 2.05%. Fatty acid composition expressed as a percentage of seed weight is shown in Appendix Table 3. The top selection according to linoleic acid was WC–S3, linolenic acid was SB–25, oleic acid was OD–12, palmitic acid was WC–S1 and stearic acids was R–15.

1.1.3.2. Effect of Site and Year on Fatty Acid Profiles

Fatty acid composition was determined for sea buckthorn oil extracted from seeds collected at two sites over three years (Appendix Table 4). Collections were made from three trees at each site. Fatty acid composition was determined using Gas Chromatography (GC). The analysis was conducted by the Crop Utilization Section,

Agriculture and Agri-Food Canada, Saskatoon. Generally seed oil from the Francis site had the highest levels of linoleic acid and stearic acid whereas the Dafoe site had higher palmitic, oleic, and linoleic acid levels. The 1997 growing season produced the highest levels of linoleic acid. The highest level of linolenic acid was in 1999. Overall, site and seed collection year had little impact on fatty acid composition.

1.1.3.3. Seed Oil Improvement

Physio-chemical properties of sea buckthorn seeds (primarily oil content) were determined using proton magnetic resonance (NMR) spectroscopy. Seed oil quality in oilseed crops and horticultural plants is known to be genetically controlled, which is expressed in the oil composition of each seed. Because sea buckthorn is a dioecious species, individual seeds form different plants in a population or community, and seeds collected from the same plant are expected to be genetically diverse, and therefore, heterogeneous for desirable oil composition. This variation can be utilized commercially by single seed selection, followed by regeneration of plants from individual seeds that possess superior oil quality and quantity. Promising plants are then vegetatively propagated to establish orchards of superior plants. For this purpose seedlots were evaluated in a non-destructive manner. The Nuclear Magnetic Resonance (NMR) technology allows non-destructive analysis of seeds in a fraction of the time and cost of traditional gas chromatography (GC) and High Pressure Liquid Chromatography (HPLC). Procedures to optimize the quality of NMR spectra of single oilseeds have been developed by Reaney *et al.* (1997).

Seeds were collected from the 200 plants in our selection program. These seeds were analysed for seed oil quantity and quality using an NMR available at the AAFC Saskatoon Research Station. Fruit samples were collected from plants in the selection program in 1997, 1998 and 1999. Seeds were extracted from the fruit collected from each plant. Seed weights for each sample were determined prior to the NMR analysis. A total of 307 samples representing 201 selections were screened for oil content. This data can be seen in Appendix Table 5.

Year of collection appeared to have only a slight effect on seed weight and oil content. Generally selections with high seed weight or oil content retained that character over the three harvest years. Seed weight ranged from 1.24 g/100 seeds to 2.32 g/100seeds. Mean seed weight was 1.73 g/100 seeds. Oil content ranged from 7.72 to 17.79 %. Mean oil content was 14.2 %.

The seed from the top oil yielding selections were sown in containers. The seedlings will be grown in the germplasm nursery at the PFRA Shelterbelt Centre for further study, breeding and selection.

1.1.4. Characteristics of Top Selections

The objective of this study was to determine physical, chemical and nutritional properties of the top five selections identified in the project. Fruit that had been frozen at

-18^{0}C was shipped to the POS Pilot Plant in Saskatoon for chemical and nutritional analysis of pulp. In addition, seed was extracted from the top selections for determination of seed oil content and fatty acid profiles. Analysis was completed by the AAFC - Saskatoon Research Station. The results of these analyses are shown in Tables 1 and 2.

Table 1. Chemical analysis of top selections.

Source	Analysis	6476	6582	FR-14	OL-6	RC-4
	Oil Content (%)	12.2	11.1	13.9	13.8	17.4
	Fatty Acid (% seed wt)			15.4	13	17.3
	Palmitic Acid (%)			6.4	6.1	6.1
Seed	Stearic acid (%)			2.9	2.9	2.8
	Oleic acid (%)			18.7	15.3	14.1
	Linoleic acid (%)			34	34.7	36.1
	Linolenic acid (%)			36.2	39.3	39.2
	Moisture (%)	86	86.6	88	85.5	88.9
	Oil Content (%)	1.93	2.68	1.34	2.08	1.73
	Soluble Sugars (°Brix)	13.7	11.4	11.2	13.3	9.2
	Carotenes (µg/100g)					
	luteine	138.4	147.7	223.2	341.5	198.1
Pulp	alpha-carotene	349.1	260.9	964.3	594.3	762.1
&	beta-carotene	4979	5044	8871	5562	6695
Peel	lycopene	784.4	1084	1242	905.8	1538
	Tocopherols (mg/100g)					
	alpha-tocopherol (Vit	4.3	4.4	7.9	9.9	8.1
	gamma-tocopherol	<5ppm	<5pp	<5ppm	<5ppm	<5ppm
	delta-tocopherol	<5ppm	<5pp	<5ppm	<5ppm	<5ppm
Whole	**Vitamin C (mg/100g)**	192	180	57	63	68

Table 2. Description of top female selections.

Selection	Growth Habit	Fruit Wt (g/100)	Seed Wt (g/100)	Pedicel (mm)	Harvest (Index)*	Thorns
6582	Compact	84.2	2.24	5.6	5.6	Absent
FR-14	Tree-Like	65.1	2.01	7.8	3.7	Present
OL-6	Compact	53.9	2.12	4	1.9	Present
R-C4	Tree-Like	45.4	2.13	6.9	3	Present
6476-2	Compact	67.5	2.1	3.9	3.5	Absent

*Harvest Index - Higher numbers indicate easier fruit harvesting

1.1.4.1. Vegetative Propagation of Selections

In July 1999, a total of 2650 softwood cuttings were collected from 47 female and 12 male selections. The cuttings were rooted under mist in a propagation bed and then stored at -2° C until planting. In the beginning of February, 2000 the rooted cuttings were placed in the greenhouse to resume growth. Bud break and the growth of cuttings was recorded for each selection three times per week for a period of one month. The majority of cuttings broke the bud 7-10 days after they were placed in the greenhouse.

In 2000, a total of 1,900 softwood cuttings were collected from 28 female and one male selection. Cuttings were collected the last week of June and the first week of July. The cuttings were dipped in IBA rooting hormone and rooted under mist in a propagation bed. Rooting was complete within six weeks of sticking. The cuttings were allowed to harden and then stored at -2° C. The cuttings were moved into the greenhouse in mid March, 2001 for second flush of growth.

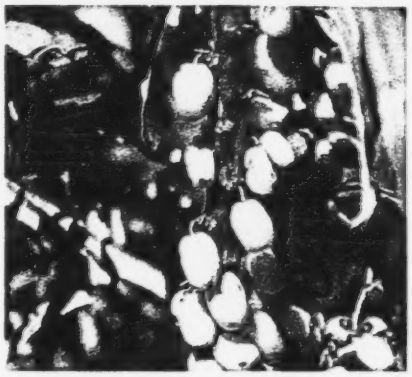
In 2001, a total of 840 softwood cuttings were collected from 18 female selections. Cuttings were collected the last week of June and the first week of July. The cuttings were dipped in IBA rooting hormone and rooted under mist in a propagation bed with bottom heat at 22°C. Rooting was complete within six weeks of sticking. 11-48-0 fertilizer was applied to the cuttings in weeks 1-4 and 20-8-20 fertilizer was applied in weeks 5-8. The cuttings were moved into the greenhouse in mid February, 2002 for second flush of growth.

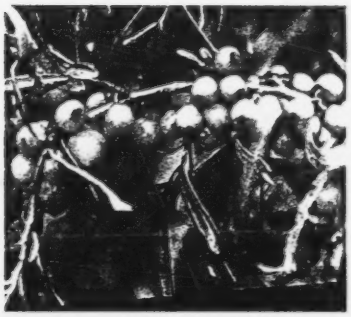
1.1.5. Cultivar Release

In 2000, male and female selections were conditionally released to commercial nurseries in Saskatchewan, Manitoba and Alberta. These selections were released for preliminary increase and evaluation of propagation techniques, including tissue culture. Participating nurseries include Prairie Plant Systems in Saskatoon, D'nA Gardens in Elnora, AB and Day Spring Nurseries in Teulon, MB. Plant material will be provided to

other nurseries as interested. The following cultivars developed in the project are being released to commercial nurseries in the prairies.

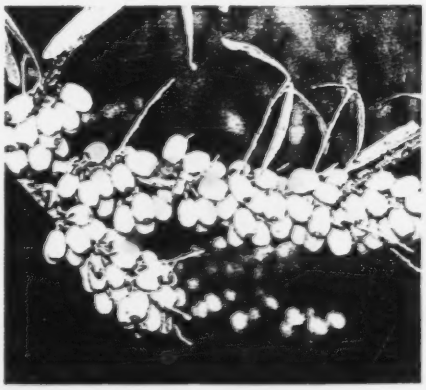
Selection 6582 - selected from a seedling population of *Hippophae rhamnoides* ssp. *mongolica* obtained from Russia in the mid 1990's. The selection is virtually thornless, has good yields (2.3 kg/plant in the first year of fruiting) of large fruit that is easily harvested by hand or shaking. Berries are large (0.8 g), yellow-orange with a pedicel length of 5-6 mm. Form is compact and easily trained to a single stem. Fruit is sour sweet with soluble sugar content of 11.4 °Brix. Vitamin C content is 181 mg/100g. Seed oil content is 11.1 percent.



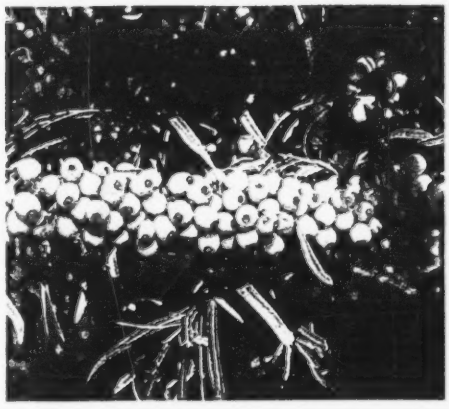


Selection FR-14 - selected from a seedling population of Indian-Summer (*Hippophae rhamnoides* ssp. *mongolica*) growing in a field shelterbelt near Francis Saskatchewan. The selection has thorns and good yields of fruit. Berries are large (0.65 g), yellow-orange with long pedicels (6-7 mm), which makes the fruit relatively easy to harvest by hand or shaking. Form is compact and easily trained to a single stem. Fruit is sour sweet with soluble sugar content of 11.2 °Brix. Seed oil content is 13.9 percent.

Selection Ol-6 - selected from a seedling population of Indian-Summer (*Hippophae rhamnoides* ssp. *mongolica*) growing in a field shelterbelt at the Canada-Saskatchewan Irrigation Centre at Outlook, Saskatchewan. The selection has thorns and high yields of fruit. Berries are medium (0.54 g), yellow-orange with medium length pedicels (3-4 mm). Fruit can be harvest by hand or shaking. Form is compact and easily trained to a single stem. Fruit has a high soluble sugar content of 13.3 °Brix. Seed oil content is 13.8 percent. Pulp oil content is 2.1 percent.

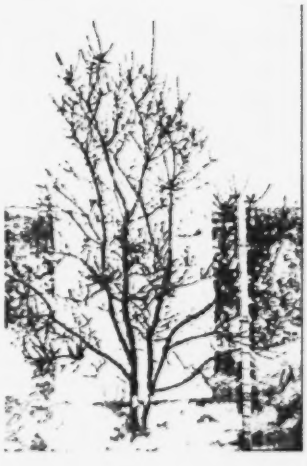


Selection R-C4 - selected from a seedling population of Indian-Summer (*Hippophae rhamnoides* ssp. *mongolica*) growing in the Rafferty wildlife habitat planting near Estevan, Saskatchewan. The selection has thorns and good yields of fruit. Berries are medium (0.45 g), yellow-orange with long pedicels (6-7 mm). Fruit can be easily harvested by hand or shaking. Form is tree-like and easily trained to a single stem. Fruit has a medium soluble sugar content of 9.2 °Brix. Seed oil content high at 17.4 percent. Pulp oil content is 1.7 percent. This selection has high flavonoid concentrations in both the fruit and leaves.



Selection 6476 - selected from a seedling population of *Hippophae rhamnoides* ssp. *mongolica* originating from Russia. The selection is virtually thornless, has good yields of large fruit that is easily harvested by hand or shaking. Berries are large (0.67g), yellow-orange with a pedicel length of 3-4 mm. Form is compact and easily trained to a single stem. Fruit has a soluble sugar content of 13.7 °Brix. Vitamin C content is 184 mg/100g. Seed oil content is 11.1 percent. Pulp oil content is 1.9 percent.

Selection H6-121 – a male tree selected from a seedling population of Indian-Summer (*Hippophae rhamnoides* ssp. *mongolica*) growing in a hedge row at the AAFC-PFRA Shelterbelt Centre. The tree grows to over 3m high with a large crown. The selection has large male buds that possess 17 flowers on the average.



1.2. Germplasm Nursery

A germplasm nursery was established in 1996 on a 1.5 hectare land at the PFRA Shelterbelt Centre, Indian Head. The nursery serves as a living collection of seed sources and clonal cultivars obtained from China and several European countries. Sixty-one accessions representing six *Hippophae* species (*H. goniocarpa*, *H. gyantensis*, *H. neurocarpa*, *H. rhamnoides*, *H. salicifolia* and *H. tibetana*) were planting in the nursery. *H. rhamnoides* accessions included four subspecies, e.g., *ssp. mongolica*, *ssp. rhamnoides*, *ssp. sinensis* and *ssp. turkestanica*. The accessions in the germplasm nursery are listed in Appendix Table 1.

1.2.1. Clonal Evaluation

Domesticated cultivars under evaluation are: Frugana, Leikora, Hergo and Pollmix from Germany and Proznachnaya and Ljubitelskaya from Latvia and Chuiskaya, Prevoskhodnaya, Dar Kartuni, Podorok Sadu, Limarnaya, Trofimovskaya, Samorodok, Rodnotchok, Velikan, Orangenaya, Obilnaya, Vitaminaya and Alei from Russia. These cultivars are being compared to cultivars developed in the project. The test is located at the AAFC-PFRA Shelterbelt Centre. It includes a total of 80 cultivars arranged in a Randomized Complete Block design with three plant plots replicated three times. Planting began in the June, 1999 and continued as new cultivars became available. The last cultivars were planted in 2001.

1.2.2. Genetic Diversity and Seed Source Study

The project has assembled the largest germplasm nursery of sea buckthorn species and subspecies in North America. This germplasm nursery consists of approximately 500 plants from six subspecies; including *rhamnoides, sinensis, turkestanica, gyantensis, neurocarpa* and *mongolica.*

Data collection from the germplasm nursery consisted of placing each individual tree into one of three classes (e.g. small, medium or tall) for each of the following characters: height, fruit size and colour (for mature female trees), pedicel length, thorniness, leaf size, leaf loss, leaf colour, leaf pubescence, canopy width and suckering habit (Table 3).

Table 3. Characters examined for data collection of ssp. *rhamnoides* and ssp. *sinensis*.

Characters Examined
Height
Fruit Size
Fruit Colour
Pedicel Length
Thorniness
Leaf Size
Leaf Loss
Leaf Colour
Leaf Pubescence
Canopy Width
Suckering Habit

Fifty-one *sinensis* and 60 *rhamnoides* trees were tested. The test was confined to these two subspecies, as they were the only ones with a large enough population to produce a significant test. Diversity indices were then determined for each character within both subspecies, with a maximum possible diversity index of 1.099. Phenotypic diversity for each character within both subspecies was estimated using the Shannon-Weaver information index and a t-test was used to determine significant difference from the maximum diversity index. A chi-square test was then performed to determine significant differences between diversity indices of the two subspecies for each character (e.g. are the differences between the diversity indices for height for *rhamnoides* and *sinensis* significantly different from one another?)

Subspecies *rhamnoides* was more diverse than ssp *sinensis* for seven of the 11 characters, but was less diverse for the remaining four (Table 4). Overall, ssp *rhamnoides* was more diverse than ssp. *sinensis* (0.734 *versus* 0.652 Whereas the diversity indices for only five characters were significantly lower than the theoretical maximum in *rhamnoides*, in ssp. *sinensis* the indices were lower than the theoretical maximum value of 1.099 (Table 5).

The frequency distributions of the two subspecies,*rhamnoides and sinensis* were compared for the 11 characters given in Table 3 using the ususal chi-square tests. The chi-square estimates, given in Table 6, show that the distributions were similar for six of the 11 characters.

Table 4. Diversity index for 11 characters of two subspecies of sea buckthorn at the germplasm nursery at the PFRA Shelterbelt Centre, Indian Head, Saskatchewan.

Character	Diversity Indices	
	ssp. *rhamnoides*	ssp. *sinensis*
Height	0.891	0.684
Fruit Size	0.878	0.604
Fruit Colour	0.658	1.051
Pedicel Length	1.046	0.906
Thorniness	0.746	0.774
Leaf Size	0.679	0.684
Leaf Loss	0.086	0.328
Leaf Colour	0.819	0.670
Leaf Pubescence	0.824	0.224
Canopy Width	0.815	0.974
Suckering Habit	0.628	0.271
Average	0.734	0.652

Table 5. Z values for comparison with the theoretical maximum diversity index for 11 characters of two subspecies of sea buckthorn at the germplasm nursery at the PFRA Shelterbelt Centre, Indian Head, Saskatchewan.

Character	t-Test Estimate for Variance	
	ssp. *rhamnoides*	ssp. *sinensis*
Height	-1.0798	-2.6490*
Fruit Size	-0.7041	-2.0965*
Fruit Colour	-1.6981*	-0.1741
Pedicel Length	-0.1708	-0.7067
Thorniness	-1.9730*	-1.8595*
Leaf Size	-2.9020*	-2.6490*
Leaf Loss	-6.9426*	-4.1161*
Leaf Colour	-1.5554	-2.7207*
Leaf Pubescence	-1.6150	-5.1985*
Canopy Width	-1.7200*	-0.6643
Suckering Habit	-1.3117	-2.4642*

Significant at P = 0.05

Table 6. Chi-square estimate for frequency distribution for 11 characters of two subspecies of sea buckthorn at the germplasm nursery at the PFRA Shelterbelt Centre, Indian Head, Saskatchewan.

Character	X^2 estimate for frequency distribution
Height	40.01*
Fruit Size	8.169
Fruit Colour	6.191
Pedicel Length	6.268
Thorniness	15.36*
Leaf Size	2.548
Leaf Loss	2.445
Leaf Colour	28.36*
Leaf Pubescence	17.60*
Canopy Width	33.85*
Suckering Habit	1.197

* Significant at P = 0.05

In an earlier study, Jana and Schroeder (2000) compared genetic diversity of sea buckthorn grown in Saskatchewan with that of three other species of the family Elaeagnaceae: buffaloberry (*Shepherdia argentia* Nutt.), Russian Olive (*Elaeagnus angustifolia* L.) and silverberry (*Elaeagnus commutata* Bernh.). Sea buckthorn was found to be very diverse. This high diversity will allow sea buckthorn to meet the adaptive challenges presented by the harsh prairie environments and help its adaptation as a succesful agroforestry species.

Jana, S., W.R. Schroeder. 2000. Sea Buckthorn: A Near-Ideal Plant for Agroforestry on the Canadian Prairies. *Journal of Sustainable Forestry*. Vol. 10, No. 3 / 4, 2000, pp. 335-339.

2.0. Sea Buckthorn Propagation

2.1. Softwood Cutting Rooting Study

The objective of this study was to examine the rooting of softwood sea buckthorn cuttings in different rooting media. The media tested included peat moss, perlite and vermiculite in combination or alone and Metro-Mix (a pre-mixed media consisting of peat moss, vermiculite and perlite). The peat moss was buffered with lime to have a pH of 6.0. The media was lightly packed in Hillson root trainer containers and then watered until evenly moist. Softwood cuttings (10-13 cm long) were collected from a single sea buckthorn plant July 5, 1999. Leaves were stripped off the bottom 2/3 of the cutting. The cuttings were dipped in 0.5% IBA immediately prior to sticking in the media. The study was conducted in a propagation bed with bottom heat (22°C). During the study mist was applied to the cuttings for 10 seconds every 25 minutes. The study was arranged in a Randomized Complete Block Design with four, eight cutting replications per treatment. The treatments tested are as follows:

1. Peat moss:Perlite (1:1)
2. Peat moss:Perlite (2:1)
3. Peat moss:Perlite (3:1)
4. Peat moss:Perlite (1:2)
5. Peat moss:Perlite (1:3)
6. Peat moss:Vermiculite (1:1)
7. Vermiculite
8. Metro-Mix

After 10 weeks, the cuttings were removed from the media and the number of cuttings rooted, number of roots per cutting, dry weight of roots per cutting recorded. Data was analyzed using ANOVA. Data means are shown in table 5.

Table 7. Rooting of sea buckthorn softwood cuttings in different media.

Treatment	Rooting (%)	Roots (No./Cutting)	Total Root Weight (Wt/Cutting - gms)
Peat moss:Perlite (1:1)	97	11	0.047
Peat moss:Perlite (2:1)	97	15	0.040
Peat moss:Perlite (3:1)	100	10	0.038
Peat moss:Perlite (1:2)	100	15	0.054
Peat moss:Perlite (1:3)	97	15	0.039
Peat moss:Vermiculite (1:1)	100	14	0.045
Vermiculite	100	18	0.039
Metro-Mix	100	15	0.072

Rooting percentage and number of roots per cutting of treatments did not differ significantly, however the dry weight of roots per cutting showed significant differences

between treatments. The total root weight per cutting was highest for cuttings rooted in Metro-Mix. The other treatments had similar total root weight and did not differ significantly. All the media tested provided a suitable rooting medium for sea buckthorn. Because Metro-Mix is readily available and requires no preparation, it is the recommended media for rooting sea buckthorn softwood cuttings.

2.2. Seed Germination

Sea buckthorn seed germination may be affected by length of storage and pre-treatment. Normally seed is soaked in water at 20°C for 48 hours, then air-dried prior to sowing. An electronic vibration technique reported to improve seed germination and vigour has been developed in China and was available for testing in Canada. The objective of the study was to determine the effect of electronic vibration on germination and vigour of sea buckthorn seed stored for one and ten years.

Sea buckthorn seed collected in 1990 and 1999 was used in the study. Seed had been stored at -10°C in sealed poly bags. Seeds were sown in flats filled with soil/peat media. The treatments tested for each seed collection year were: 1. Water soak for 48 hours at 20°C followed by vibration; 2. No water soak, vibration; 3. No water soak and no vibration; 4. Water soak only. Seeds were sown on June 16, 2000. The study was arranged in a randomized complete block design with 8 replications. Each plot consisted of 25 seeds. Germination percentage was determined after 30 days.

Seed treatments were sown on June 16, 2000. The number of seeds germinated was recorded every 3 days beginning June 26 and concluding July 17, 2000. The final seed germination is shown in Table 6. Germination percentage ranged from 75.5 to 85.0 percent. Seed germination was not significantly affected by length of storage or by seed treatment. There were no significant interactions between length of seed storage and seed pre-treatment. Sea buckthorn seed can be stored for long periods with no significant decline in germination capacity. Seed will germinate equally well if sown with no pre-treatment, soaked for 48 hours or vibrated.

Table 8. Results of seed germination study.

Seed Collection Year	Seed Treatment	Germination (%)
1990	Soak + Vibration	79.5
	Vibration only	82.0
	No Soak, No Vibration	85.0
	Soak Only	84.5
1999	Soak + Vibration	82.5
	Vibration only	75.5
	No Soak, No Vibration	82.0
	Soak Only	82.0

3.0. Inter-cropping Demonstration

The 20 acre intercropping demonstration project was established in 1997. The planting tree rows are spaced 25 meters apart allowing for agriculture production between the rows. Beginning in 1999 female sea buckthorn trees were identified and pruned. The objective of the pruning was to train branches, promote growth and facilitate harvesting. The crowns were pruned to remove overlapping branches, and long branches were cut to encourage development of lateral shoots. The goal is to allow more light penetration into the plant. Superfluous growth on the lower part of the tree trunk was removed to promote development of framework branches. The plan is to use an open centre pruning system. With this system trees are trained to a multi-leader with major branches extending up and away from the trunk, giving a vase-shaped tree. Plants pruned in this way will have higher yields and better quality fruit. This pruning method also facilitates mechanical harvesting using a hand held shaker. Pruning was repeated in 2000 and 2001. In 2001, female trees were planted to replace dead trees.

The landowner planted a forage crop between the sea buckthorn rows in 2001. The demonstration is showing the benefits integrating sea buckthorn with an agricultural crop. The rows of sea buckthorn are improving the microclimate in the protective zone of the tree row and improving moisture conditions by trapping snow and reducing evapo-transpiration during the growing season. These factors are resulting on increased forage yields. In addition the shrubs are a source of fruit which the landowner is harvesting and marketing. In 2001, the landowner harvested 1000 kg of fruit.

4.0. Sea Buckthorn Harvesting

4.1. Hand Harvesting of Indian Summer and *H. rhamnoides* ssp. *sinensis*

In recent years more than 200 acres of sea buckthorn orchards have been established in the prairie provinces. Indian Summer and *sinensis* are two types of sea buckthorn being considered for commercial orchard production in Saskatchewan. Indian-Summer sea buckthorn is a seed-propagated cultivar known for its adaptation to prairie conditions. It yields on average 3-5 kg of fruit per shrub. Berries are predominantly arranged in dense clusters distributed along two-years-old branches.

The subspecies *sinensis* was introduced from China and has become available to growers in the last three or four years. It grows vigorously, but it is very thorny. The berries are arranged individually or in small clusters firmly attached to the branches and main trunk. *Sinensis* performance is being compared to Indian Summer in test plots at the PFRA Shelterbelt Center. The objective of this study was to determine the differences in hand-harvesting efficiency between *sinensis* and Indian-Summer sea buckthorn.

Fruit samples were hand-harvested from a total of three plants per type. Selection was based on fruit firmness and yield. Harvesting was performed on three first-time-fruiting *sinensis* and three mature Indian-Summer plants with good yield and firm fruit. Hand harvesting with strippers (a handmade tool in the shape of tweezers) was performed for five minutes on each plant. Weights of the fresh fruit and a total number of berries per sample were recorded (Table 9). The force required to remove fruit was recorded for ten berries on each tree using Shimpo Digital Force Gauge, Model FGV-1A.

Table 9. Results of hand-harvesting for Indian-summer and *sinensis* sea buckthorn.

Sample No.	Sea buckthorn variety	Weight (g)	Number of berries per sample	Fruit removal force (g)
1.	sinensis	212	785	107.3
2.	sinensis	248	743	153.4
3.	sinensis	264	845	97.5
4.	'Indian-Summer'	687	1580	96.3
5.	'Indian-Summer'	505	1819	99.1
6.	'Indian-Summer'	531	1220	94.7

The results show that *sinensis* samples have, on average, 18% more berries per weight unit than Indian Summer samples. This means that on average the 'Indian-Summer' fruit is larger than *sinensis* fruit. The average weight of fruit samples collected from *sinensis* during the 5 minute test period was 241 g compared to 574 g for Indian-Summer. The force required to remove fruit was 96.7 g for Indian-Summer and 119.4 g for *sinensis*.

This preliminary study demonstrates difficulties in hand harvesting the variety *sinensis*. The difficulties in hand harvesting from *sinensis* can be explained by the small fruit size, short pedicel length, presence of large numbers of thorns along the fruiting branch and tightness which the fruit is attached to the stem. These factors will also make mechanical harvesting of *sinensis* more difficult than 'Indian-Summer'.

4.2. Evaluation of P.A.M.I. Sea Buckthorn Harvester

A prototype mechanical harvester for harvesting both leaves and fruit from the Prairie Agriculture Machinery Institute (PAMI) in Portage la Prairie, MB was tested at a sea buckthorn project site south of the Shelterbelt Centre twice during 2000. Engineers from PAMI collected data from the test harvest and made adjustments to the design of the harvester based on this data. In 2001 the harvester was again tested on a sea buckthorn site south of the Shelterbelt Centre. Testing was done twice for leaf harvest and four times for berry harvest. Engineers from PAMI again made design adjustments based on data collected from 2001. In 2001 damage from the harvesting operation was assessed.

5.0. Flavonoids in Sea Buckthorn

This three-year study was conducted by Branka Barl and Leonid Akhov of the Herb Research Program (HRP), Department of Plant Sciences, U of S. Given the potential of sea buckthorn flavonoids as a source of nutraceutical ingredients for humans and animals, flavonoids warrant further studies.

5.1. Introduction

Sea buckthorn has been used extensively for hundreds of years in folk medicine in Russia, China, Mongolia and other countries in Central Asia where it grows wild. Seed oil, fruit juice, leaves and bark are used to treat burns, stomach ulcers, cancer, heart diseases, and variety of other conditions. Sea buckthorn also grows in parts of Iran, Turkey, and Northern and Central Europe. Over the past 20 years, it has been developed into a viable nutraceutical crop in China. All aerial parts of the plant including berries, seed and leaves are processed into dozens of value-added products and are becoming increasingly popular in Asia (Japan and China), Europe (Russia, Ukraine, Finland, Sweden, Germany, Italy, Poland) and to a limited extent in North America.

Sea buckthorn berries, seed, and leaves are marketed in form of food (juice, tea, nutritional drink), dermatological products (ointments and oils) and dietary supplements (extracts, concentrates) worldwide. Sea buckthorn berries are an excellent source of unsaturated fatty acids, macro- and micro-nutrients, particularly vitamins C, E (tocopherols), A (carotenoids), phytosterols and flavonoids. Sea buckthorn seeds have up to 20% oil and are rich in unsaturated fatty acids with well documented health benefits. In contrast to sea buckthorn berries and seeds, very limited studies on natural compounds such as flavonoids from leaves have been conducted. Leaf flavonoids were studied in Russia in 1970's and more recently in China, but have not been addressed in North America in any significant way. Given that flavonoids are documented to exhibit a

variety of biological activities, such as anti-inflammatory, anti-oxidative, anti-viral, adaptogenic, anti-tumor and immunostimulatory, flavonoid content and composition is a desirable trait that warrants future considerations in developing selections of sea buckthorn for Saskatchewan.

While scientific literature on sea buckthorn in English language is limited, it abounds in Russian and Chinese language. Over 100 European and several American patents have been registered to date. High volume of patent literature suggests great potential for value-added processing opportunities for sea buckthorn, which could be exploited in the near future, given an estimated 200 acres under sea buckthorn in Saskatchewan. This also suggests a great need for reliable and well documented scientific information on nutraceutical properties of various plant parts of sea buckthorn, particularly selections that are mainly grown in Saskatchewan. Moreover, it is important to investigate feasibility of applying various cost-effective processing technologies on all plant parts of sea buckthorn. Such activity would support knowledge based development of new products with unique and well-defined application/s for niche and mass markets.

This study addresses flavonoids in berries and leaves as natural health ingredients with potential applications in food, botanical and cosmetic preparations. Flavonoids can be marketed as medicinal ingredients in form of tablets, capsules and concentrated extracts, as nutraceutical ingredients in form of teas, nutritional drinks, and bars, and as a cosmetic ingredients in various skin products. We determined the total flavonoid content in leaves and berries of Indian Summer selections, and in a *Hippophae* germplasm nursery maintained at the PFRA Shelterbelt Centre in Indian Head. We also investigated processing conditions suitable for recovery of flavonoids in the form of aqueous extracts, analytical method for the separation and quantification of specific flavonoid compounds, and isolation and structure determination of main flavonoid compounds in leaves of Indian Summer selections.

Flavonoid extracts from Indian Summer selections released for preliminary increase and evaluation of propagation techniques, such as FR-14, RC-4, and OL-6, and the flavonoid compounds isolated in this study are being investigated for its antioxidative potential and other biological activities by the Herb Research Program, University of Saskatchewan.

5.1. Total Flavonoid Content in Sea Buckthorn Leaves and Berries

Objective To determine total flavonoid content in samples of sea buckthorn leaves and berries provided by PFRA Shelterbelt Centre in Indian Head, SK, over a three-year period (1999 to 2001) using non-specific spectroscopic method.

Materials and Methods

Samples A total of 75 sea buckthorn samples were received in 1999, 50 samples of dry leaves and 25 samples of frozen berries. A total of 72 sea buckthorn samples were received in 2000 (48 leaves and 24 berries) and 78 samples in 2001 (68 leaves and 10

berries). Summaries of the samples received during the 3-year period are presented in Table 10 and 11. Dry leaves were stored in a dry, dark place at 10°C and fruit was stored at –20°C prior to analysis.

Total Flavonoids Total flavonoids were determined according to Hedin *et al.* (1992). Dried leaves were ground in coffee grinder and passed through a 24-mesh sieve. Berries were lyophilized and gently crushed with a pestle to facilitate seed removal; seed-free berries were ground in a blender prior to chemical analyses.

Samples were extracted with 50% (v/v) methanol at 40°C over 2 hours in a water bath shaker. An aliquot of each extract was centrifuged at 12, 000 RPM for 15 minutes and analyzed for the total flavonoid content in triplicate. Hedin's method is based on chemical derivatization of flavonoids with diphenyl borinic acid ethanolamine ester and absorbance measurement of the colored product at 440 nm. Since flavonoids in sea buckthorn were expected to be chiefly isorhamnetin derivatives, a calibration curve was generated using isorhamnetin in the concentration range of 1-38 mg/mL; consequently, flavonoid content is expressed in isorhamnetin equivalents.

Instrumentation Spectrophotometric analysis was conducted using a Spectronic 1001+spectrophotometer, Milton Roy (USA).

Table 10. Sea buckthorn species and varieties analyzed for flavonoids in leaves (1999-2001).

No. of Accessions	Accession	Location/Species
Indian Summer		**Location**
1	BAL-7	Balcarres
1	TB-2	Dafoe
2	FR-13, FR-14	Francis
7	M-1, M-7, M-10, M-14, M-16, M-19, M-22	Melville
2	OL-1, OL-6	Outlook
5	R-38, R-41, R-43, R-L, R-C4	Rafferty
4	H6-5A, H6-35, H6-50, N4-19	SBC-Indian Head
1	SB-26	Summerberry
1	WS-S4	
Accessions from germplasm nursery		**Species**
1	EP6468	*H. gyantensis* (China)
1	EP6469	*H. neurocarpa* (China)
1	EP5789	*H. monogolica* (Chifend, China)
8	EP: 6476,6477,6478,6479,6480, 6582, 6483,6485	*H. rhamnoides rhamnoides* (Russia, Finland, SK)
9	EP: 6455, 6456, 6457, 6458, 6459#1, #2, #3, 6459 MIX, 6463	*H. rhamnoides sinensis* (China, Finland)
7	EP: 6464, 6465, 6466, VNIALMI, POLMIX, LEIKORA, HERGO	*H. rhamnoides turkestanica* (China, Finland)
Total 51		

Table 11. Sea buckthorn species and varieties analyzed for flavonoids in berries (1999-2001).

No. of Accessions	Accession	Location/Species
Indian Summer		**Location**
1	BAL-7	Balcarres
1	TB-2	Dafoe
2	FR-13, FR-14	Francis
7	M-1, M-7, M-10, M-14, M-16, M-19, M-22	Melville
2	OL-1, OL-6	Outlook
5	R-38, R-41, R-43, R-L, R-C4	Raffety
2	H6-35, N4-19	SBC-Indian Head
1	SB-26	Summerberry
1	WC-S4	
Accessions from germplasm nursery		**Species**
4	EP6459#1, #2, #3, #4	*H. rhamnoides sinensis* (China)
1	EP-6582	*H. rhamnoides rhamnoides* (Russia)
Total 27		

Results and Discussion

Total flavonoid content of Indian Summer leaves and leaves of several accessions from *Hippophae* germplasm nursery from Indian Head is presented in Table 12 and 13, respectively. Total flavonoid content of Indian Summer berries and berries from *Hippophae* germplasm collection are shown in Table 14. Flavonoid content, expressed as isorhamnetin equivalents, was in the range of 0.83% (EP6455) to 2.00% (R-C4) for air-dried leaves, and 0.18% (H6-35) to 0.56% (EP6459, #3) for lyophilized (freeze-dried) seed-free berries. The highest flavonoid content was found in leaves of the following 5 selections of Indian Summer: R-C4 (2.00%), OL-6 (1.78%), FR-13 (1.71%), M-16 (1.64%), and M-14 (1.59%), in a single accession of *H. rhamnoides mongolica* EP5789 (1.68%), and 3 accessions of *H. rhamnoides sinensis* EP 6456 (1.59%), EP 6459 MIX (1.58%), and EP 6458 (1.59%).

The 5 best quality selections of Indian Summer contained 1.56 to 2.00% flavonoids in dry leaves and 0.35 to 0.49% in lyophilized fruits. As in 1999, selection R-C4 Rafferty contained the highest amount of flavonoids in both leaves (2.00%) and fruits (0.49%), followed by FR-13 Francis, OL-6 Outlook, M-16 Melville, and OL-1 Outlook. These results are in the good agreement with the first year data suggesting that flavonoid accumulation is reproducible. The correlation coefficient between the total flavonoid content of samples collected in 2000 and 1999 is 0.912. From the germplasm collection, the *H. rhamnoides sinensis* (EF 6456, EP6459 MIX) and *H. rhamnoides mongolica* (EP5789) contained the highest amount of flavonoids in leaves, 1.58%, 1.54% and 1.53% respectively. However, for the second consecutive year, flavonoid content was lower than

in most promising selections of Indian Summer. Flavonoid content of leaves and berries, harvested in 2001, was considerably lower than in previous two years. That can partly be explained by the drought conditions in 2001. In addition, it was noticed that for 13 samples (namely: EP6468, EP5789, EP6483, EP6455, EP 6457, EP 6458, EP 6459, EP 6464 and HERGO) from germplasm nursery the level of total flavonoids decreased from year to year. Mainly all these plants were obtained from China. Meanwhile, the plants, which obtained from Finland and Russia, except the sample EP 6483, showed stable level of total flavonoids.

Table 12. Total flavonoid content in sea buckthorn leaves of accessions from *Hippophae* germplasm nursery in Indian Head, SK (1999-2001).

				Total Flavonoid Content[1] (%w/w)				
Sea buckthorn Species	**Subspecies**	**Accession**	**Source**	*1999*	*2000*[2]	*2001*[2] *July*	*August*	*September*
H. gyantensis		EP6468	China (PRC)	1.60	1.50 ±0.15		1.22 ±0.01	
H. neurocarpa		EP6469	(PRC)	1.35	1.35 ±0.07	1.23±0.01	1.43±0.05	
H. rhamnoides	*mongolica*	EP5789 (4-1)	Chifeng, (PRC)	1.68	1.53 ±0.02	1.18±0.05	0.90±0.01	
H. rhamnoides	*mongolica*	EP6476	Russia	1.13	1.03 ±0.05	1.15±0.01	0.96±0.01	
H. rhamnoides	*rhamnoides*	EP6477	Finland	1.01	0.99 ±0.05	1.07±0.01	1.03±0.04	1.14±0.06
H. rhamnoides	*rhamnoides*	EP6478	Finland	0.96	0.92 ±0.02	0.85±0.05	0.78±0.01	0.77±0.05
H. rhamnoides	*rhamnoides*	EP6479	Saskatoon	1.21	1.07 ±0.03	1.14±0.06	1.17±0.06	
H. rhamnoides	*rhamnoides*	EP6480	Finland	0.95	0.92 ±0.04	0.95±0.05	0.77±0.03	
H. rhamnoides	*rhamnoides*	EP6482	Russia			1.16±0.05		1.21±0.01
H. rhamnoides	*rhamnoides*	EP6483	Finland	1.26	1.17 ±0.06	1.03±0.05	0.99±0.01	
H. rhamnoides	*rhamnoides*	EP6485	Finland	1.07	1.12 ±0.04	1.08±0.03	0.89±0.06	
H. rhamnoides	*sinensis*	EP6455	Wushan, PRC	1.42	1.26 ±0.05	0.83±0.09	0.98± 0.05	
H. rhamnoides	*sinensis*	EP6456	PRC	1.62	1.58 ±0.04			
		(1-2)					1.34±0.01	1.28±0.01
		(1-3)				1.07±0.03		
		(1-1)					1.59±0.01	
H. rhamnoides	*sinensis*	EP6457	Liancheng, PRC	1.58	1.50 ±0.03	1.03±0.04	1.21±0.01	
H. rhamnoides	*sinensis*	EP6458	Liancheng, PRC	1.59	1.53 ±0.02			
		(1-2)				1.28±0.05		
		(1-3)					1.18±0.04	
H. rhamnoides	*sinensis*	EP 6459 #1	Liancheng, PRC	1.42	1.39 ±0.01	0.95±0.01	1.06±0.01	1.13±0.01
H. rhamnoides	*sinensis*	EP 6459 #2	Liancheng, PRC	1.25	1.29 ±0.04	0.94±0.03	1.01±0.01	
H. rhamnoides	*sinensis*	EP 6459 #3	Liancheng, PRC	1.54	1.45 ±0.01	0.99±0.01	1.05±0.01	
H. rhamnoides	*sinensis*	EP 6459 #4	Liancheng, PRC	1.38		1.08±0.01	0.96±0.01	
H. rhamnoides	*sinensis*	EP 6459 MIX	Liancheng, PRC	1.58	1.54 ±0.03	0.97±0.01	0.91±0.01	
H. rhamnoides	*sinensis*	EP6463	Finland	1.32	1.31 ±0.03	1.28±0.01	1.15±0.02	
H. rhamnoides	*turkestanica*	EP6464	Kongliu, PRC	1.48	1.45 ±0.02	0.94±0.01	0.84±0.02	
H. rhamnoides	*turkestanica*	EP6465	Chabuchar, PRC	1.51	1.53 ±0.02	1.46±0.01		1.47±0.01
H. rhamnoides	*turkestanica*	EP6466	Finland	1.24	1.18 ±0.02	1.17±0.04	1.18±0.01	
		VNIALMI		1.43	1.42 ±0.02	1.26±0.01		
		POLMIX		1.37	1.52 ±0.03	1.26±0.02		
		LEIKORA		1.11	1.06 ±0.03	0.94±0.01		
		HERGO		1.43	1.37 ±0.01	1.22±0.01		

[1]Expressed in isorhamnetin equivalents; [2]Flavonoid content is expressed as a mean±standard deviation, n=3.

Table 13. Total flavonoid content of Indian Summer sea buckthorn leaves collected in Saskatchewan (1999-2001).

Accession	Source	Total Flavonoid Content[1] (%w/w)				
		1999	*2000*[2]	*2001*[2]		
				July	*August*	*September*
BAL-7	Balcarres	1.52	1.09 ±0.02			
TB-2	Dafoe	1.39	1.25 ±0.02			
FR-13	Francis	1.68	1.71 ±0.02		1.34±0.05	
FR-14	Francis	1.34	1.31 ±0.03		1.13±0.01	
M-1	Melville	1.54	1.40 ±0.01			
M-7	Melville	1.59	1.46 ±0.03			
M-10	Melville	1.53	1.44 ±0.03			
M-14	Melville	1.59	1.54 ±0.01			
M-16	Melville	1.64	1.57 ±0.03			
M-19	Melville	1.52	1.46 ±0.03			
M-22	Melville	1.34	1.33 ±0.02			
0L-1	Outlook	1.50	1.56 ±0.02			
0L-6	Outlook	1.78	1.63 ±0.03		1.44±0.01	
R-38	Rafferty	1.24	1.26 ±0.03		1.15±0.02	
R-41	Rafferty	1.59	1.34 ±0.01			
R-43	Rafferty	1.56	1.42 ±0.00			
R-L	Rafferty	1.32	1.22 ±0.00			
R-C4	Rafferty	2.00	1.89 ±0.00		1.70±0.01	1.62±0.01
H6-5A	SBC	1.54				
H6-35	SBC	1.60	1.45 ±0.01	1.29±0.00	1.39±0.00	
H6-50	SBC	1.21	1.06 ±0.00	0.84±0.00	1.12±0.01	
N4-19	SBC	1.14	1.19 ±0.01	1.22±0.01		
SB-26	Summerberry	1.37	1.31 ±0.01			
WS-S4						1.16±0.01

[1]Expressed in isorhamnetin equivalents.

[2]Flavonoid content is expressed as a mean±standard deviation, n=3.

5.2. Extraction of Sea Buckthorn Leaf and Berry Flavonoids in Water

Objective To establish feasibility of recovering flavonoids from sea buckthorn leaves and berries in form of an aqueous extract, a preferred type of extract for food and related applications.

Materials and Methods

Samples: Samples of Indian Summer leaves and berries of accessions R-C4 (Rafferty), provided by the PFRA Shelterbelt Centre in Indian Head was used in this experiment.

Table 14. Total flavonoid content in lyophilized sea buckthorn berries of Indian Summer and accessions from *Hippophae* germplasm nursery in Indian Head, SK (1999-2001).

Accession	Source	Total Flavonoid Content[1] (%w/w)			Average Single Berry Weight (g)	Average Single Berry Size (mm)
		1999	2000	2001	2001	2001
Accessions from the germplasm nursery						
EP 6459 #1	Liancheng, PRC	0.30	0.26	0.45	0.27	7.1
EP 6459 #2	Liancheng, PRC	0.30	0.25	0.45	0.17	6.1
EP 6459 #3	Liancheng, PRC	0.42	0.36	0.56	0.28	7.2
EP 6459 #4	Liancheng, PRC	0.29				
EP-6582	Russia			0.18	0.53	11.1
Indian Summer						
BAL-7	Balcarres	0.42	0.38			
TB-2	Dafoe	0.27	0.25			
FR-13	Francis	0.30	0.26	0.26	0.31	8.6
FR-14	Francis	0.16	0.29	0.13	0.51	10.8
M-1	Melville	0.37	0.33			
M-7	Melville	0.37	0.31			
M-10	Melville	0.30	0.26			
M-14	Melville	0.27	0.22			
M-16	Melville	0.36	0.35			
M-19	Melville	0.30	0.24			
M-22	Melville	0.35	0.32			
0L-1	Outlook	0.36	0.32			
0L-6	Outlook	0.37	0.31	0.29	0.35	10.3
R-38	Rafferty	0.24	0.20	0.19	0.49	10.8
R-41	Rafferty	0.48	0.35			
R-43	Rafferty	0.42	0.34			
R-L	Rafferty	0.27	0.22			
R-C4	Rafferty	0.49	0.41	0.18	0.37±0.010	9.9
H6-35	SBC	0.20	0.18			
N4-19	SBC	0.25	0.21			
SB-26	Summerberry	0.42	0.36			
WC-S4				0.45	0.27±0.011	10.0

[1]Expressed in isorhamnetin equivalents.

Extraction procedure: Ten grams of frozen R-C4 seabuckthorn berries (seeds removed) and 0.5 g of leaves were crushed in the blender and extracted successively three times with 50 mL of H_2O in the water bath shaker at 30°C to simulate juicing operation. Then, plant material was extracted three times with 50 mL of 80% ethanol (50°C, shaker), followed with two times extraction with 50 mL of the mixture of methanol : formic acid : water (50:1:49 v/v) under the same conditions. Each extraction procedure lasted for 2 hours. Following each extraction, plant material was filtrated through a paper tissue under vacuum to separate extract from plant residue.

Each extract was analyzed for the total flavonoid content using a non-specific spectroscopic method (Hedin's method) and was subjected to flavonoid profiling using HPLC method.

Results and Discussion

In a laboratory, flavonoids are usually extracted with aqueous methanol or pure methanol as a solvent. This method gives high extraction recovery, but can not be used in a food production system because of high methanol toxicity. The results of our experiments (Table 15) revealed that flavonoids could largely be extracted with a highly polar solvent such as water; flavonoid concentrations in water and methanol extracts were comparable. In a given experiment, about 85% of leaf flavonoids were extracted in a single step; a total of 96% flavonoids were extracted by a 3-step water extraction. Additional extraction with ethanol recovered about 4% of remaining less polar flavonoids. The case was similar with berries. About 79% of flavonoids were extracted during first water extraction, and about 92.6% by three-step water extraction. Berries contained higher amount of ethanol insoluble substances, 2.3 μg/mL than leaves, which contained 0.2 μg/mL.

The flavonoid profile of methanol and water extracts of seabuckthorn leaves (R-C4), presented in Figure 1 and 2, revealed minor differences in composition. For example, in leaves, compound with the retention time 23 min was more abundant in water than in methanol extract, while compounds with retention times of 18 min and 31 min were more abundant in methanol extract. Minor difference were also observed between profiles of water and methanol extracts of berries (Fig. 3 and 4).

In summary, our bench micro-scale experiments suggested that water extraction of both seabuckthorn leaves and berries at 30 °C , which simulates typical fruit juicing operations, is feasible. Over 90% of flavonoids could be recovered and lyophilized for potential pharmaceutical, nutraceutical or food applications. Fine selection of optimal extraction parameters is still required.

Table 15. The concentration of flavonoids in different extracts of seabuckthorn leaves and berries of Indian Summer accession Rafferty R-C4.

Sample No.	Type of Extract	Flavonoids Concentration[1] (μg/mL)	
		Leaves	Berries
1	50% methanol extract (control)	189.3	75.2
2	First water extract	190.5	69.1
3	Combined second and third water extracts	26.5	12.3
4	Combined two ethanol extracts	9.4	4.2
5	Combined two methanol extracts	0.2	2.3

[1]Expressed as isorhamnetin equivalent.

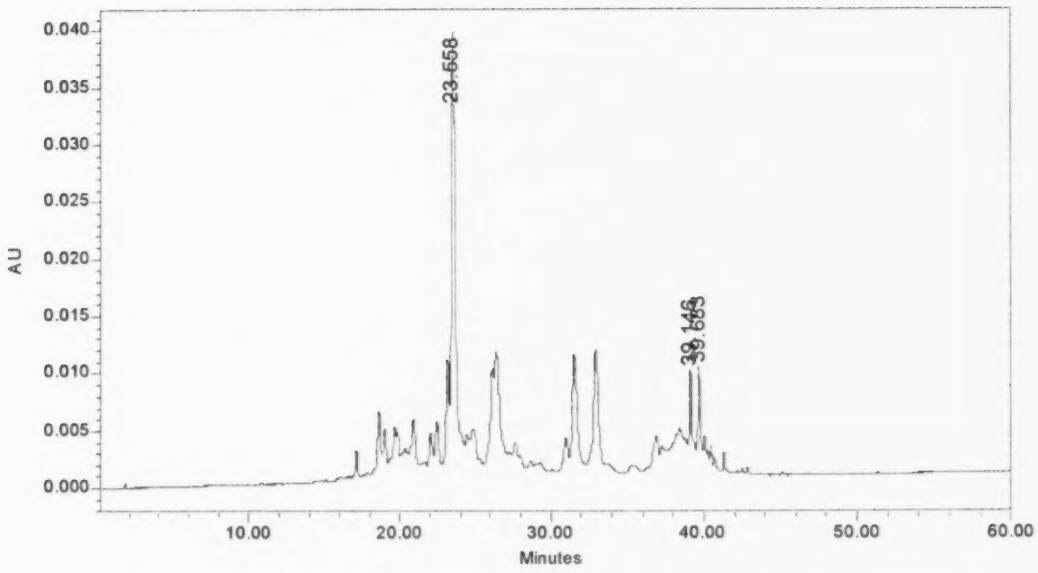


Fig. 1. Typical HPLC profile of sea buckthorn leaves water extract of Indian Summer selection R-C4 (356 nm).

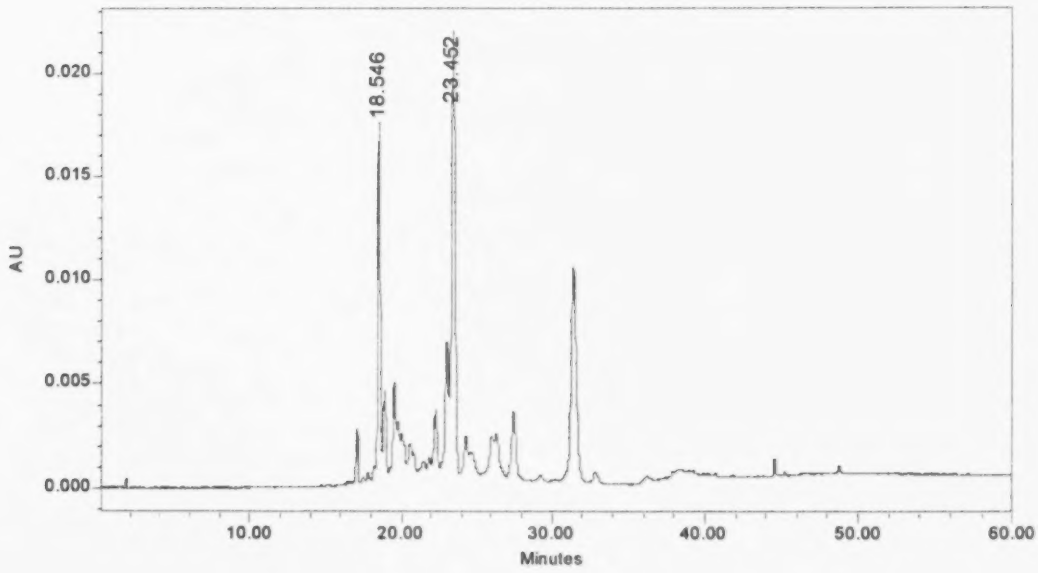


Fig. 2. Typical HPLC profile of sea buckthorn leaves methanol extract of Indian Summer selection R-C4 (356 nm).

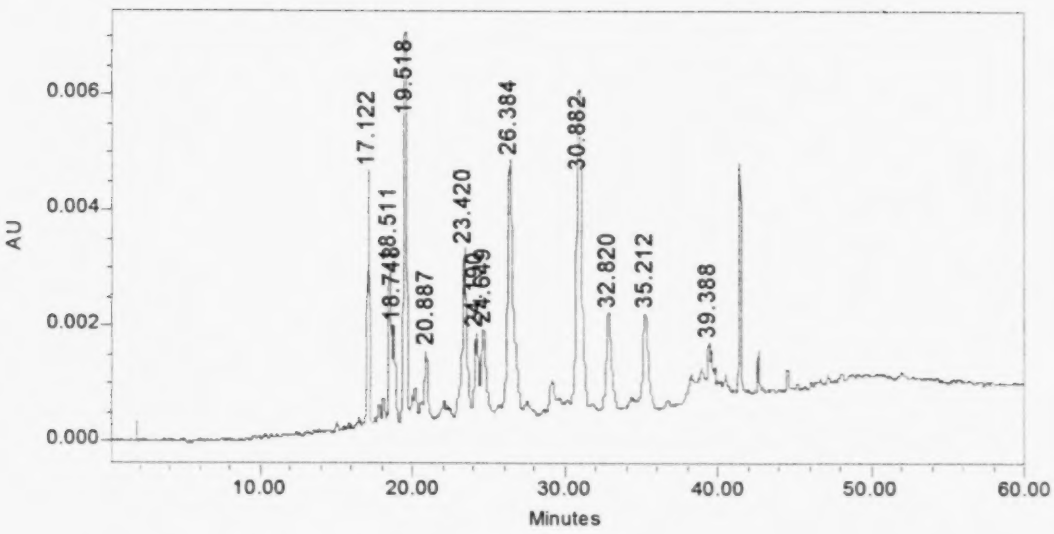


Fig. 3. Typical HPLC profile of sea buckthorn berries water extract of Indian Summer selection R-C4 (356 nm).

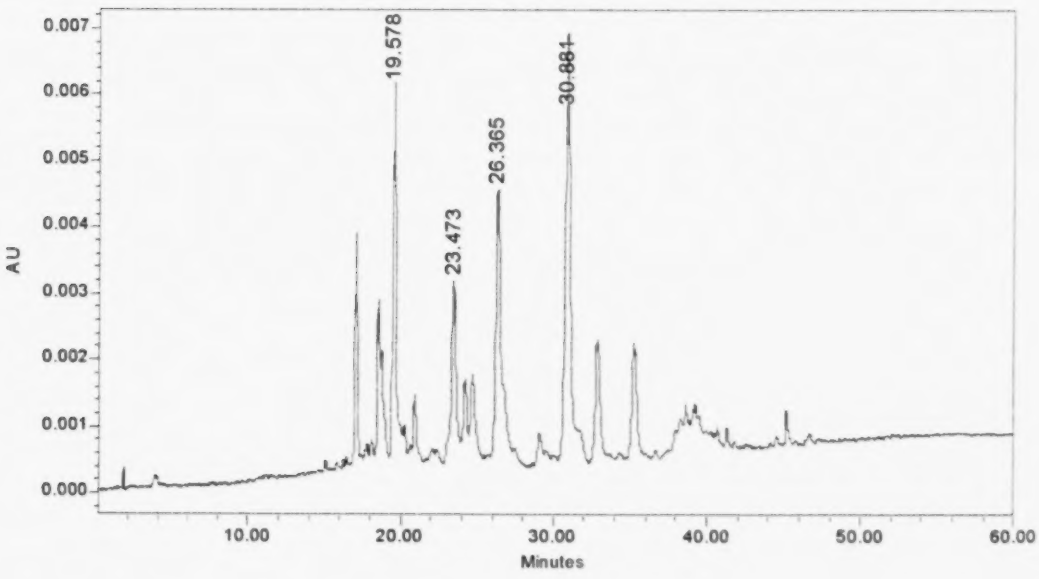


Fig. 4. Typical HPLC profile of sea buckthorn berries methanol extract of Indian Summer selection R-C4 (356 nm).

5.4. Composition of Flavonol Glycosides in Sea Buckthorn Leaves and Berries

Objectives To develop a chromatographic method for simultaneous separation of flavonoids and flavonoid aglycones in seabuckthorn leaves and berries (1); to investigate flavonoid composition of leaves of sea buckthorn Indian Summer and from selected accessions from *Hippophae* germplasm nursery in Indian Head.

Materials and Methods

Samples A total 11 samples of Indian Summer leaves from accessions H6-35, Fr-13, R-38, H6-50, OL-6, Fr-14, R-C4 and N4-19 as well as 4 samples of leaves from *Hippophae* germoplasm nursery, namely *H. rhamnoides sinensis* (Finland), *H. rhamnoides mongolicas* (China), *H. neurocarpa* (China) and *H. gyantensis,* (China) harvested in 2001 were provided by the PFRA Shelterbelt Centre in Indian Head. Dry leaves were stored at dark,dry place at 10°C prior to analysis.

Extraction Samples were extracted with 50% (v/v) methanol at 40°C over 2 hours in a water bath shaker. An aliquot of each extract was centrifuged at 12, 000 RPM for 15 minutes prior to testing. The methanolic extracts were investigated by HPLC.

HPLC Analysis Liquid chromatographic analysis was performed with a Waters liquid chromatography system comprised of a Waters 600 system controller, Waters 996 diode array detector, Water 717 Plus autoinjector and a Waters Symmetery Shield™ RP_{18} (5µm, 3.9x150 mm) column. A linear gradient program shown in Table 15. For HPLC analysis of aglycone, a linear gradient program, described by Brolis (1998) was used. Peaks were detected at 354 nm. Data was collected and processed digitally with Millenium[32] chromatography software.

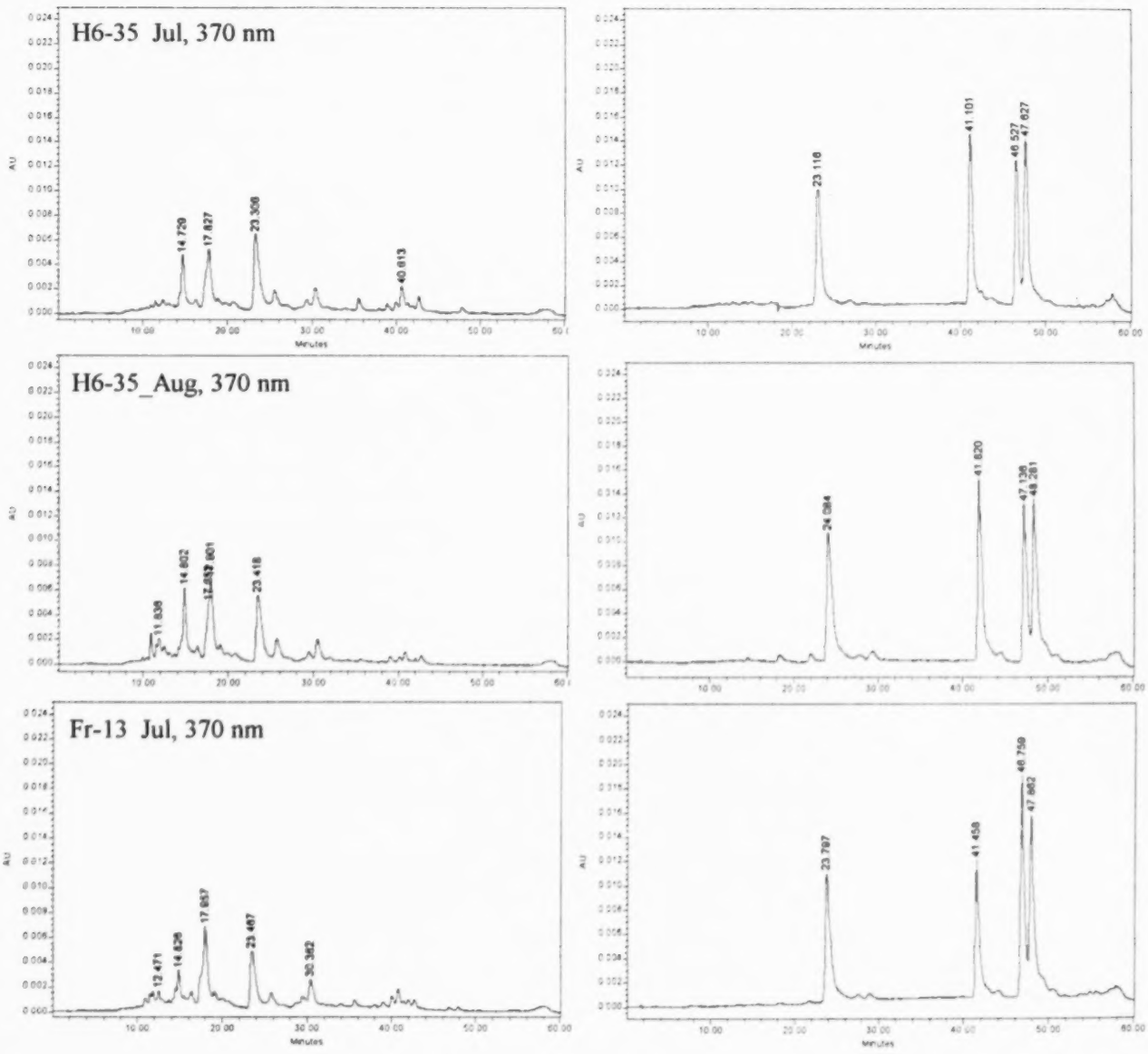
Hydrolysis Analysis. Samples of extracts (3-4 mg) were transferred to 2-mL vials with screw caps and 0.5 mL of 2 N HCl was added to each vial. The vials were heated in the oven at 85°C for 60 minutes. For HPLC analysis of aglycone the samples were diluted three times with water and loaded on Waters Sep-Pak C-18 (1 g) cartridge. The cartridge was washed with 10 mL of water to remove hydrochloric acid. The aglycones were eluted with 2 mL of MeOH. The methanolic eluates were investigated by HPLC.

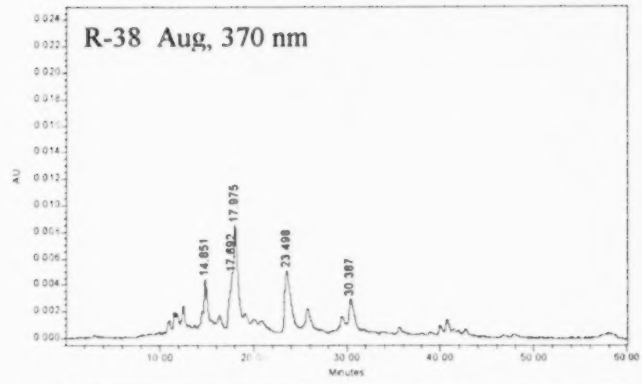
Table 16. Gradient program for RP-HPLC analysis.

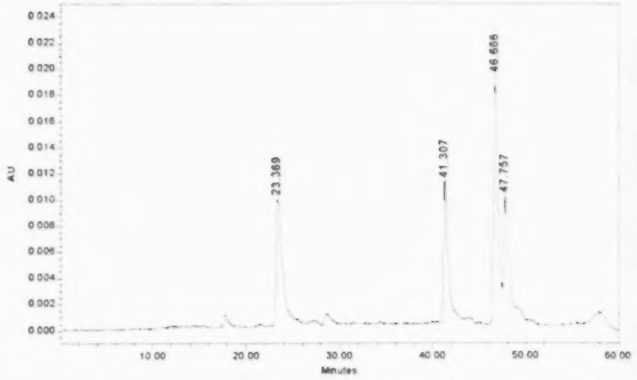
Time (min)	Solvent A[1] (%)	Solvent B[2] (%)	Solvent C[3] (%)
Initial	100	0	0
10	85	15	0
30	70	20	10
40	10	75	15
55	5	80	15
56	100	0	0
65	100	0	0

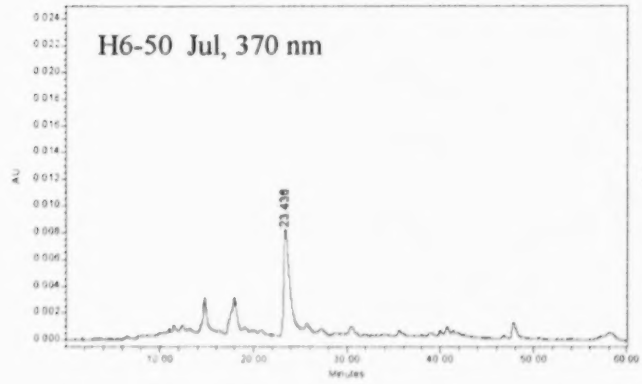
[1]Solvent A = Water – 85% phosphoric acid (99.7:0.3 v/v)
[2]Solvent B = Acetonitrile; [3]Solvent C = Methanol

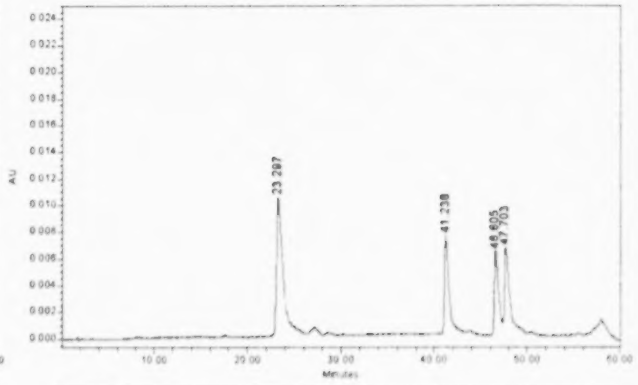
Figure 5. HPLC profile of sea buckthorn leaves methanol extract of nine Indian Summer selections.

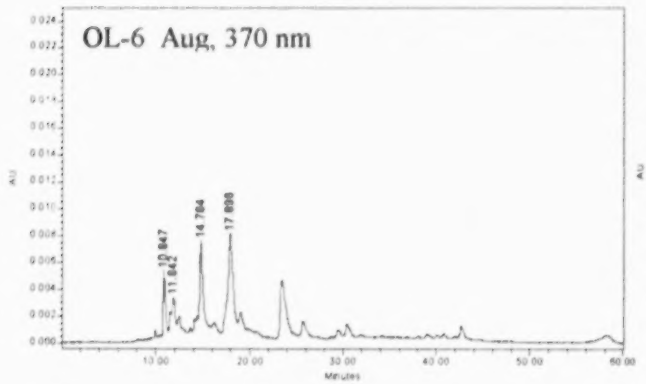


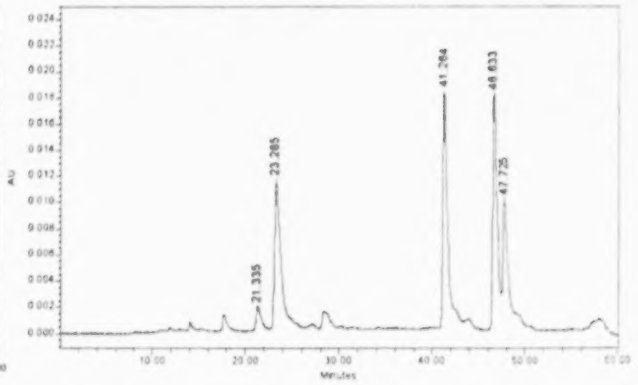

R-38 Aug, 370 nm
14.651
17.492
17.975
23.498
30.387
AU
Minutes

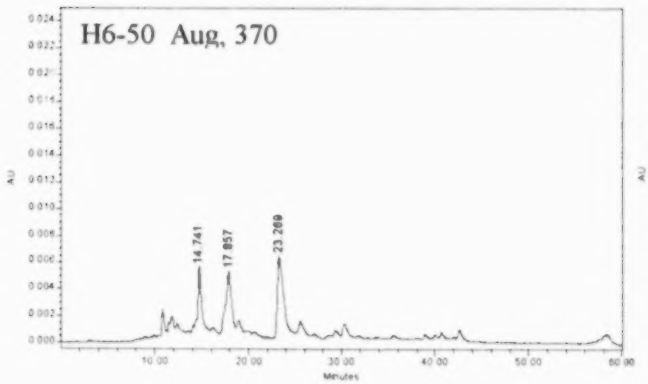

23.369
41.307
46.666
47.757
AU
Minutes

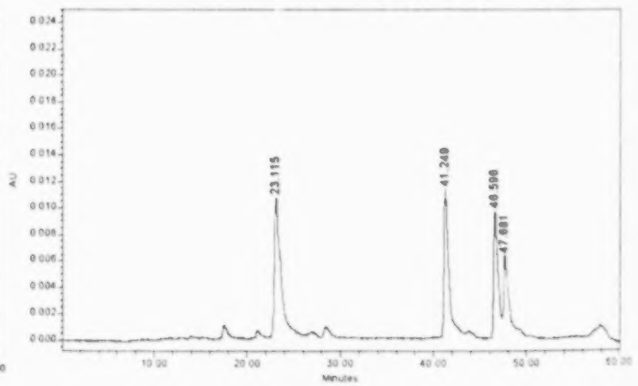

H6-50 Jul, 370 nm
23.436
AU
Minutes

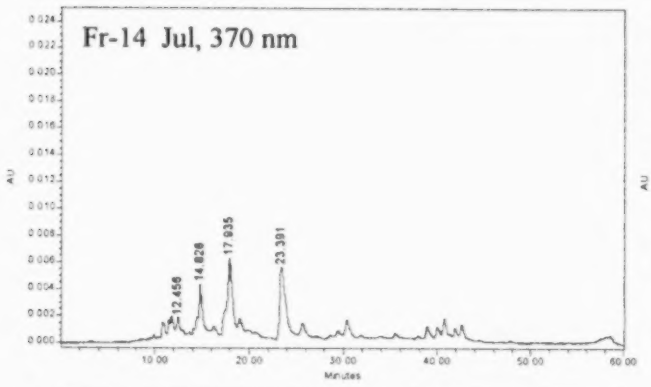

23.297
41.238
46.605
47.703
AU
Minutes

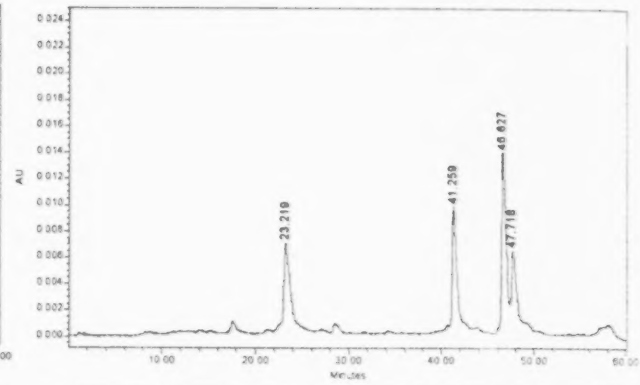

OL-6 Aug, 370 nm
10.847
11.842
14.704
17.898
AU
Minutes

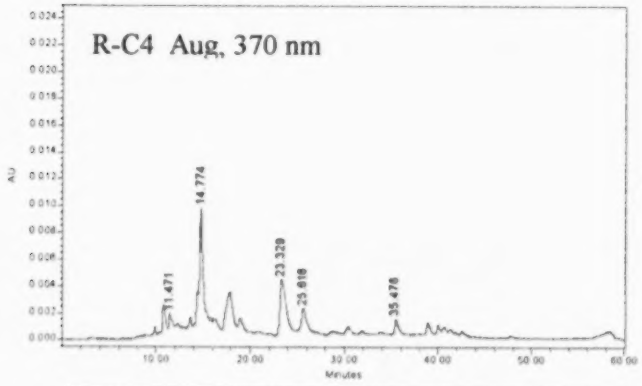

21.335
23.285
41.264
46.633
47.725
AU
Minutes

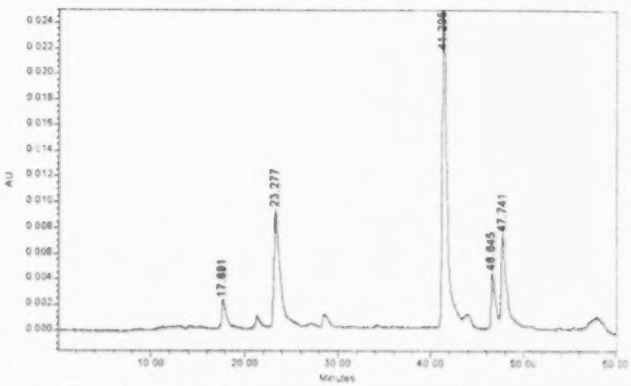

H6-50 Aug, 370
14.741
17.857
23.269
AU
Minutes

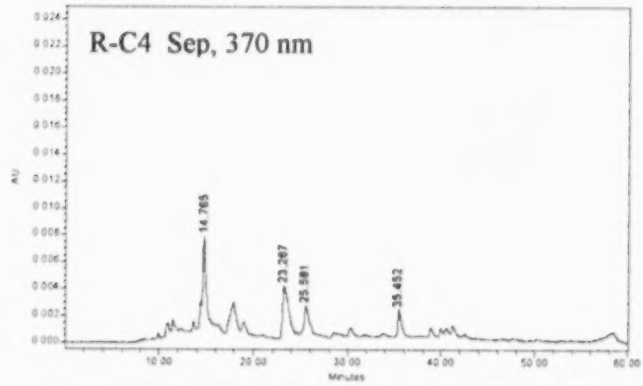

23.115
41.249
46.598
47.681
AU
Minutes

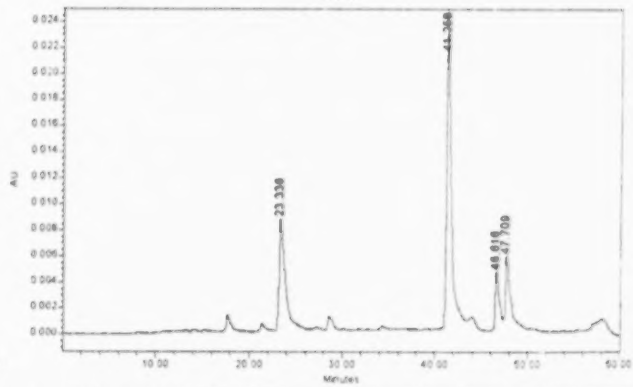

Fr-14 Jul, 370 nm
AU
Minutes
12.456
14.826
17.935
23.391

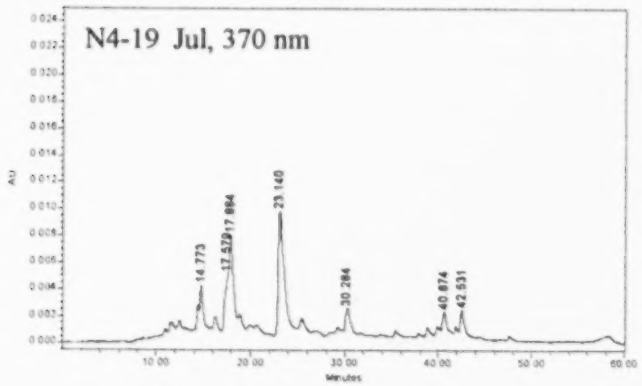

AU
Minutes
23.219
41.259
45.627
47.718

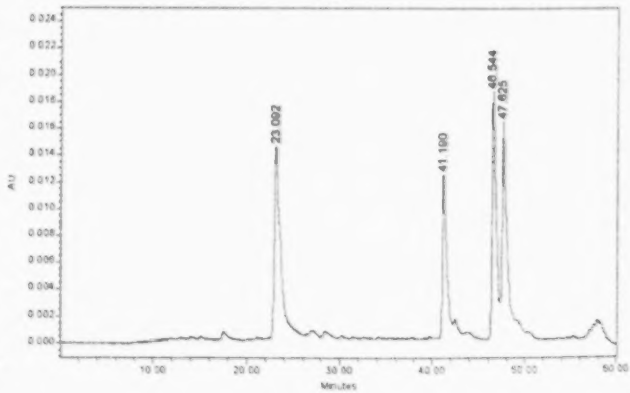

R-C4 Aug, 370 nm
AU
Minutes
11.471
14.774
23.329
25.619
35.478


AU
Minutes
17.691
23.277
41.206
48.845
47.741


R-C4 Sep, 370 nm
AU
Minutes
14.765
23.267
25.581
35.452


AU
Minutes
23.338
41.358
48.618
47.709


N4-19 Jul, 370 nm
AU
Minutes
14.773
17.884
23.140
30.284
40.874
42.531


AU
Minutes
23.092
41.180
46.544
47.625

Quercetin, isorhamnetin and kaempferol with retention time 41.2 min, 46.6 min and 47.7 min, respectively, were found in the hydrolized methanol extracts of leaves of all Indian Summer selections tested. These flavonoids were identified by the retention times of appropriate commercial standards by HPLC. The ratio between three aglycones obtained after hydrolysis of the sea buckthorn leaves extracts in nine Indian Summer selections is shown in Table 17. As shown in Table 17, the ratio between quercetin and kaempferol varied from 1:0.1 for accession R-C4 to 1:2 for accession R-38.

Table 17. The ratio between three aglycones obtained after hydrolysis of the sea buckthorn leaves extracts[1].

Sample I.D.	Quercetin	Isorhamnetin	Kaempferol
H6-35_July	1	0.9	1.0
H6-35_Aug	1	0.9	0.9
Fr-13_July	1	0.8	0.6
R-38_Aug	1	2.0	0.9
H6-50_July	1	0.9	0.8
H6-50_Aug	1	0.9	0.4
Ol-6_Aug	1	1.1	0.5
Fr-14_July	1	1.7	0.6
R-C4_Aug	1	0.1	0.2
R-C4_Sept	1	0.1	0.1
N4-19_July	1	1.7	1.4

[1]Determined by HPLC as percentages of peak area.

The data revealed that composition of flavonoid aglycones and flavonoid glycosides varies greatly between Indian Summer selections, suggesting that flavonoid accumulation is likely genetically and environmentally controlled. Given that sea buckthorn was propagated by seed in early years after introduction to Saskatchewan, certain degree of genetic variability between Indian Summer selections is to be expected. Specifically, the ratio between quercetin and kaempferol varied from 1:0.1 for accession R-C4 to 1:2 for accession R-38.

HPLC profiles of the methanolic extracts of sea buckthorn leaves of four samples from *Hippophae* germoplasm nursery are presented in the Figure 6.

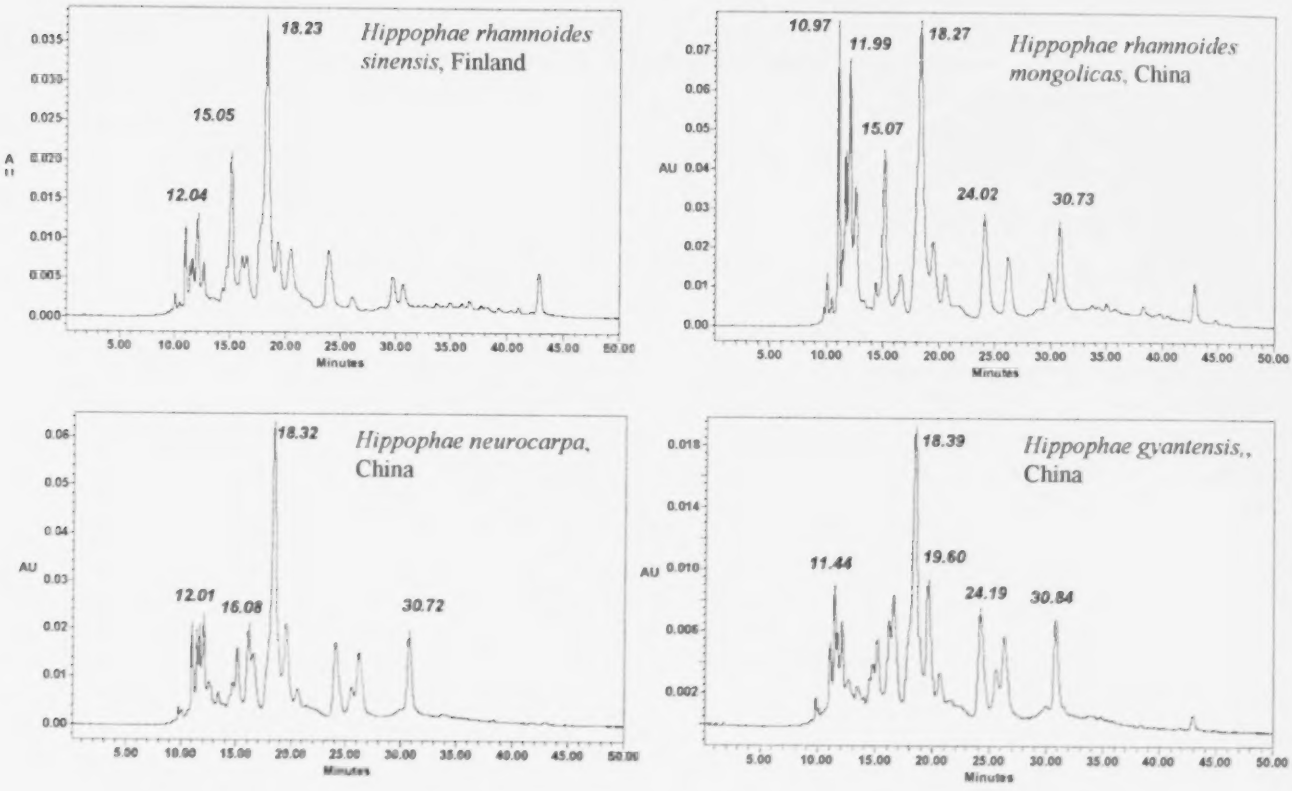


Figure 6. HPLC profile of sea buckthorn leaves extract of four species/subspecies from *Hippophae* germoplasm nursery in Indian Head, SK.

5.5 Isolation of Flavonol Glycosides from Sea Buckthorn Leaves

Objective The objectives of our study were to isolate and structurally characterize the main flavonoid compounds in leaves of two accessions (namely Rafferty and Francis), grown in Saskatchewan.

Materials and Methods

Plant Material. Flavonoids were extracted from leaves of sea buckthorn (*Hippophae rhamnoides* ssp. *mongolica*) accessions Rafferty and Francis provided in December 2000 by the PFRA Shelterbelt Center in Indian Head. Leaves were ground in coffee grinder, passed through a 24-mesh sieve and stored at 10°C prior to isolation studies.

Extraction and Isolation of Glycoside Fraction by Preparative LC. Sea buckthorn leaves (30 g) were extracted under reflux in 500 mL of 30% MeOH for 2 hours. This procedure was repeated six times. The extracts were combined and evaporated under vacuum to completely remove methanol and loaded on a short (7x14 cm) column, packed with 60 g

of Silica Gel 60 RP-18, according to procedure described by Oleszek (1988). The column was eluted with distilled water, followed by 5%, 30% and 100% MeOH. Most flavonoids were eluted with 30% MeOH. Upon evaporation of a solvent, a crude flavonoid mixture was obtained. Individual compounds from this mixture were separated by column chromatography on RP C-18 (LiChroprep, 25-40 μm, EM Science). The flavonoid mixture, extracted from the leaves of *H. rhamnoides mongolica*, accession Rafferty, was dissolved in 50 mL of 0.3% H_3PO_4 and loaded on a column (3x40 cm) packed with 60 g of RP-silica gel. The column was eluted with 200 mL of acidic buffer (0.6 mL/min), and then with 1 L of 0-15%v/v linear acetonitrile gradient, followed with 500 mL of 15% v/v acetonitrile in 0.3% H_3PO_4 (acidic buffer). The 7-mL fractions were collected with a fractor collector and monitored by TLC. Fractions showing similar TLC profile were combined. This procedure yielded four fractions, each contained a single major compound (**1-3** respectively). Flavonoid mixture from accession Francis was loaded on Waters Sep-Pak C18 10 gram cartridge. The cartridge was eluted with 200 mL of 20% v/v acetonitrile in 0.3% H_3PO_4. The 4-mL fractions were collected and fractions showing similar TLC profile were combined. This procedure yielded two fractions, one of which contained major compound **4**. Collected fractions were diluted twice with water and loaded on Waters Sep-Pak C18 1 gram cartridge. The cartridge was washed with 5 mL of water to remove acid and then flavonoid glycosides were eluted with 100% methanol. The fractions obtained after this procedure were evaporated to dryness under vacuum.

Chromatography (TLC and HPLC). TLC analyses were carried out on Merck cellulose plates, developed with 15 % v/v acetic acid in water. Plates were stained by spraying with natural products-polyethylenglycol reagent (NP/PEG) and flavonoids were detected under UV (λ=366nm) and visible light. For analysis of sugars, cellulose plates were developed with ethyl acetate/pyridine/acetic acid/water (36:36:7:21 v/v) and sprayed with anilin-phtalate reagent. Liquid chromatographic analysis was performed with a Waters liquid chromatography system comprised of a Waters 600 system controller, Waters 996 diode array detector, Water 717 Plus autoinjector and a Waters Symmetery Shield^{TM} RP_{18} (5 μm, 3.9x150 mm) column. A linear gradient program shown in Table 15 has been used. For HPLC analysis of aglycone, a linear gradient program, described by Brolis (Brolis, 1998) was used. Peaks were detected at 354 nm. Data was collected and processed digitally with Millenium^{32} chromatography software.

Spectral Analyses. Spectrophotometric analysis was conducted using a Spectronic 1001+spectrophotometer, Milton Roy (USA). The FAB-MS (both positive and negative mode) was registered with a Fison 70SEQ spectrometer using glycerol or m-nitrobenzyl alcohol as the matrix. The NMR spectra in CD_3OD were recorded with a Bruker 2D experiment: ^{1}H-^{1}HDQF-COSY (double quantum filtered direct shift correlation spectroscopy), inverse-detected ^{1}H-^{13}C HSQC (heteronuclear single quantiom coherence) and HMBC (heteronuclear multiple bond connectivity) were obtained using UX-NMR software.

Hydrolysis. Samples (3-4 mg) were transferred to 2-mL vials with screw caps and 0.5 mL of 2 N HCl was added to each vial. The vials were heated in the oven at 85°C for 60 minutes. For HPLC analysis of aglycone, the samples were diluted three times with water

and loaded on Waters Sep-Pak C-18 (1 g) cartridge. The cartridge was washed with 10 mL of water to remove hydrochloric acid. The aglycones were eluted with 2 mL of MeOH and the methanolic eluates were analyzed by HPLC. For sugars analysis after the hydrolysis procedure, aglycones were first separated from sugars through a selective extraction of water layer with 1 mL of ethyl acetate. Upon evaporation of the acid, carbohydrates were dissolved in a minimum volume of MeOH and investigated by TLC.

Results and Discussion

The HPLC profile of methanolic extracts of Indian Summer sea buckthorn leaves, accessions Francis FR-13 and Rafferty RC-4, before and after hydrolysis is shown on Figure 7. The procedure described above enabled us to isolate four compounds: two quercetin glycosides with the retention time 11.0 min and 15.1 min, and two isorhamnetin glycosides with retention time of 18.3 min and 26.2 min. Flavonoid composition and relative content in Indian Summer leaves of accessions Raferty RC-4 and Francis FR-13 is presented in Table 18.

Table 18. Flavonoid composition of sea buckthorn leaves extract[1].

Retention time of compounds (min)	Structure of isolated compounds	Relative content of compounds in *H. rhamnoides mongolica* (Raffety RC-4); %	Relative content of compounds in *H. rhamnoides mongolica* (Francis FR-13); %
11.0	#1: quercetin 3,7-O-Glu	17.8	4.5
15.1	#2: quercetin 3-Glu,7-Rha	30.4	12.7
18.3	#3: isorham 3-Glu, 7-Rha	3.8	50.3
26.0	#4: isorham 3-Glu	2.2	6.3

[1]Determined by HPLC analysis as a percentages of peak area

The structures of flavonol glycosides isolated from the leaves of sea buckthorn are shown in Figure 8. Quercetin 3,7-O-diglucoside is the dominant flavonol in the leaves of Rafferty RC-4 and accounts for ca. 30% flavonols, followed by quercetin 3-O-glucoside - 7-O-rhamnoside which accounts for approximately 18% flavonols. Compound 3 was earlier reported in leaves of seabuckthorn by Rasputina *et al.* (1975), but not compounds #1, #2, and #4. Flavonoid extracts from Indian Summer selections released for preliminary increase and evaluation of propagation techniques, such as FR-13, RC-4, and OL-6, and the isolated compounds presented above are being studied for its antioxidative potential and other biological activities by the Herb Research Program, University of Saskatchewan.

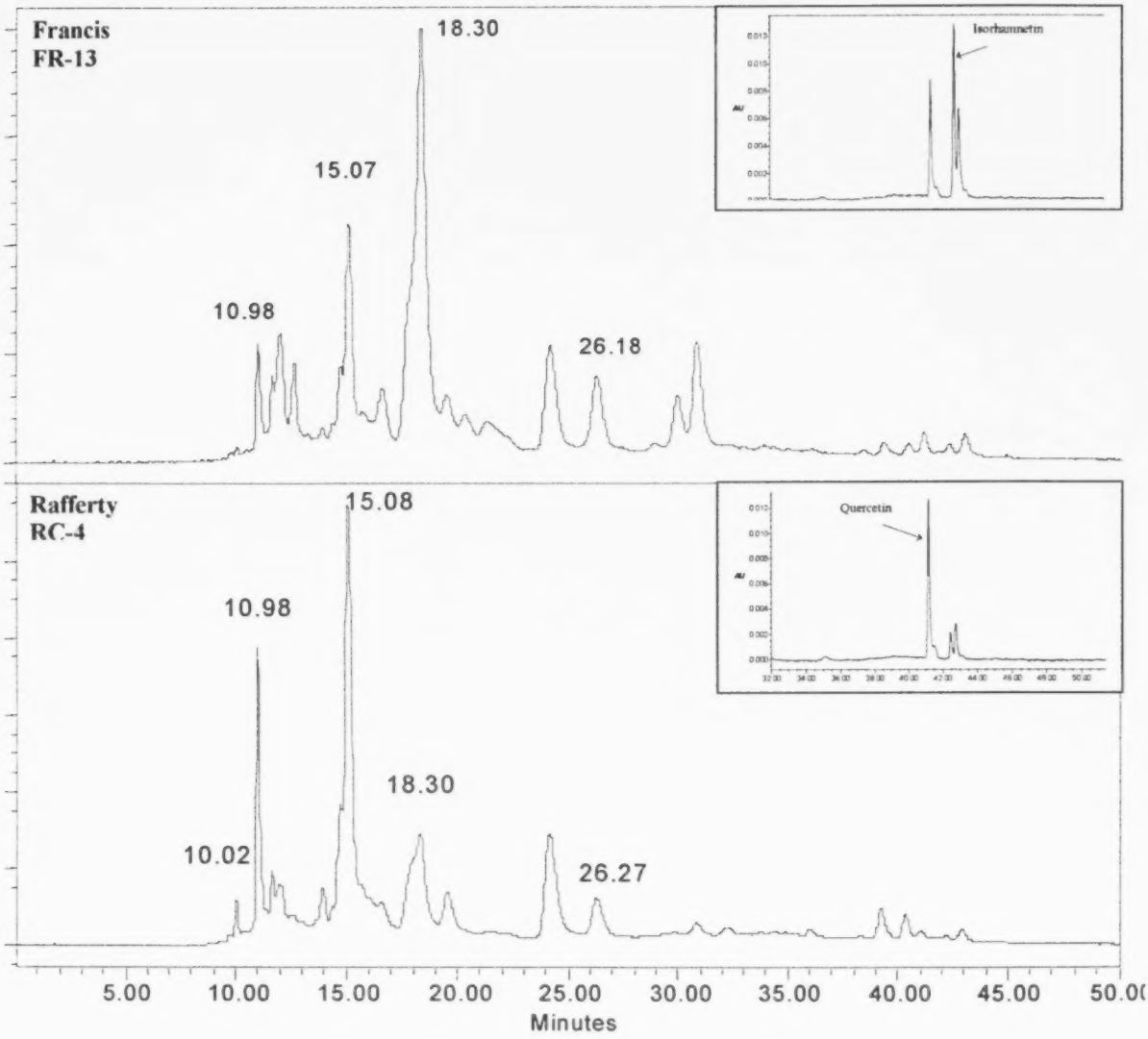
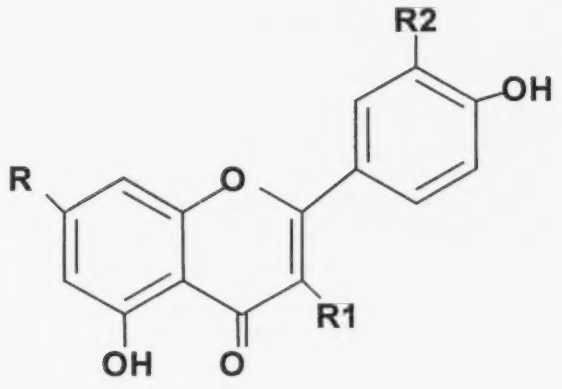


Figure 7. HPLC profile of methanol extract of Indian Summer sea buckthorn leaves, selections Francis FR-13 and Rafferty RC-4 (inserts in the right upper corner are HPLC profiles of corresponding extract upon hydrolysis, λ=354 nm).



Compound	R	R1	R2	Chemical name
1	O-glucose	O-glucose	OH	quercetin 3, 7– *O* –β-D- glucopyranoside
2	O-glucose	O-rhamnose	OH	quercetin 3- *O*-β-D-glucopyranosyl-7-O- α-L-rhamnopyranoside
3	O-glucose	O-rhamnose	OCH_3	isorhamnetin 3- *O*-β-D-glucopyranosyl-7- *O*-α-L-rhamnopyranoside
4	OH	O-glucose	OCH_3	isorhamnetin-3- *O*-β-D-glucopyranoside

Figure 8. Chemical structures of isolated flavonol glycosides.

5.6. Selected References

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D. Communication and Industry Development

1.0 Communication

The importance of communication with the public for this project cannot be underestimated. Communication was accomplished in various ways; through inquiries (including written, electronic mail, telephone and in-person), media interviews, field days, plot tours, fact sheets, journal articles, conferences and workshops.

1.1 1999 Activities

1. A Display was set up to provide information on the project for the PFRA Shelterbelt Centre field day.
2. A poster entitled, "Sea Buckthorn, it's Use in Agroforestry.", was presented at the Agroforestry – Taking Root in the West conference.
3. A poster was prepared and presented at the PFRA Managers Meeting and PFRA Technical Symposium.
4. A presentation 'Sea Buckthorn a Potential New Crop for the Prairies' was presented to interested parties at a farm meeting in Saskatoon.
5. A paper entitled, "Phenotypic Diversity in Four Woody Species on the Canadian Prairies", was published in the Canadian Journal of Plant Science. Vol. 80: 137-142.
6. A total of 225 technical inquiries were answered.

1.2 2000 Activities

1. A presentation was given at the Canada Sea buckthorn Enterprises Ltd. Annual meeting.
2. Four field and plot tours were conducted for interested parties and guests.
3. A tour was given for the Annual Investigations Tour.
4. A paper entitled, "Sea Buckthorn: A near Ideal Tree for Agroforestry", was published in the Journal of Sustainable Forestry.
5. A total of 260 technical inquiries were answered.

1.3 2001 Activities

1. A fact sheet entitled, "Sea Buckthorn A Multipurpose Plant" was developed and printed.
2. A paper entitled, "Development of Sea Buckthorn as an Agroforestry Crop on the Canadian Prairies" presented at the AFTA / PPFA Biennial Agroforestry Conference was published in the proceedings.
3. A presentation was given at the charter meeting of the Manitoba Sea Buckthorn Growers Association.
4. A Display was set up to provide information on the project for the PFRA Shelterbelt Centre field day.
5. A paper entitled, "Development of Sea Buckthorn Cultivars for Canadian Orchards" was submitted to the International Workshop on Sea buckthorn in Dehli, India.
6. Tours were given for the Saskatchewan Fruit Growers Association, the Annual Investigations Tour, Shelterbelt Field Day Tour, AFTA / PPFA Agroforestry Conference Tour, and delegations from Quebec (2) and China (2).
7. Eight field and plot tours were given to other interested parties.
8. A project update was provided to the Saskatchewan Fruit Growers Association annual field day.
9. A presentation 'The Future of Sea Buckthorn in Saskatchewan' was provided to the Saskatchewan Fruit Growers Association Annual Meeting
10. A total of 151 technical inquiries were answered.

1.4 2002 Activities

1. Several newspaper and media interviews were given.
2. A presentation 'Sea Buckthorn, a Nutraceutical Crop' was provided to the Saskatchewan Herb and Spice Association annual meeting
3. A sea buckthorn workshop was organized in co-operation with Saskatchewan Agriculture & Food and held in Indian Head, SK on March 1, 2002. It was attended by 118 people. Please see attached report on this workshop.
4. A total of 124 technical inquiries were answered.
5. Two papers were accepted for presentation at the XXVI International Horticulture Congress in Toronto, August 11-17, 2002:

B. Barl, L. Akhov, D. Dunlop, W. R.Schroeder and S. Jana: Flavonoid content and composition in leaves and berries of seabuckthorn (*Hippophae* spp.) of different origins.
L. Akhov and B. Barl. Isolation of quercetin glycosides from leaves of seabuckthorn (*Hippophae rhamnoides* ssp. *mongolica*).

1.4.1 Sea Buckthorn Workshop – March 1, 2002

A workshop on sea buckthorn was held in Indian Head, SK on March 1, 2002. The purpose of the day-long workshop was to disseminate information to producers and interested parties that has been compiled during the past six years of the ADF sea buckthorn improvement project. Topics included an international perspective on sea buckthorn, orchard management, an overview of the ADF sea buckthorn project, new cultivars developed in the project, harvesting, processing and marketing.

Agriculture Development Fund (Saskatchewan Agriculture and Food), University of Saskatchewan, and Agriculture & Agri-Food Canada (PFRA Shelterbelt Centre) were partners and sponsors for the sea buckthorn project as well as the workshop. Various individuals and organizations were invited to put up displays for the workshop. They included, PFRA Shelterbelt Centre (Climate Change and Action Fund and Sea Buckthorn Improvement Project), Pearl Creek Farms (Melville, SK), Dayspring Nursery (Teulon, MB), Prairie Plant Systems (Saskatoon, SK) and Saskatchewan Herb and Spice Association. 119 people attended the workshop. Speakers included Tom Li (Agriculture & Agri-Food Canada, Summerland, BC), Tom Beveridge (Agriculture & Agri-Food Canada, Summerland, BC), Bill Schroeder (Agriculture & Agri-Food Canada, PFRA Shelterbelt Centre), Pam Franklin (University of Saskatchewan), Erron Leafloor (P.A.M.I., Portage la Prairie, MB), Jack Winniski (Melville, SK), Marv Fenrich (Wilkie, SK) and Ron Drohomereski (Teulon, MB).

Numerous issues surrounding sea buckthorn were addressed at the workshop. These included the importance of having access to superior cultivars for use in prairie orchards, the importance of using proper maintenance practices such as pruning and weed control, the necessity of developing a mechanical harvester and possibilities for processing and marketing a product. Discussions between speakers and audience members prompted excellent questions and proposals of solutions to many of the major issues. The vital importance of forming a Saskatchewan Sea Buckthorn Growers Group, like the ones already formed in Manitoba, British Columbia and Quebec, was expressed and plans are underway to begin this process.

2. 0 Industry Development

Sea buckthorn has attracted a great amount of interest in Canada. The project has been active in assisting the development of the industry by providing technical information developed in the project to growers and industry. In addition, at the request of processors, the project has collected and provided fruit for test marketing and pilot projects. Over the past three years many growers and potential growers received technical

support from project staff. Because sea buckthorn is a new crop, this support was often a critical factor in the establishment of an orchard.

The major impact of the project has been development of new cultivars. Most of the sea buckthorn orchards planted over the past several years consist of cultivars. The major drawbacks of seedling orchards are the variability present in the plants and the equal distribution of male and female plants. The use of seedlings results in orchards that are difficult to manage and harvest. Availability of cultivars overcomes these problems, also yield increases of 50 to 75 percent can be expected when planting the new cultivars.

The availability of new sea buckthorn cultivars with be of economic benefit to growers, nurseries and processors. New orchard technology developed in the project especially related to orchard design, pruning and harvesting will significantly reduce cost of production of sea buckthorn fruit allowing for greater profit margins by growers and increased competitiveness of the industry.

3. 0 Implications for Further Development

The development of the sea buckthorn in the Saskatchewan is approaching a critical point. There is an estimated 200 to 300 acres that have been planted to sea buckthorn. All of this acreage should be in production in two to three years. Assuming a potential yield of 5 tonnes per acre this would translate into 2,750 tonnes of fruit. The highest percentage of this fruit will have to undergo primary processing if it is to have a market. This project has completed much of the research related to cultivar development, agronomics and orchard technology. Further work is required in the areas of processing, marketing and product development, determination of chemical constituents of nutraceutical value present in sea buckthorn fruit and products. The development of a mechanical harvester as under way is critical to the success of the industry.

The sea buckthorn industry needs to be carefully monitored to determine if a processing operation is going to be developed by a private investor. If this is not going to happen producers will have to look to some alternative, such as looking for a new private investor, a market outside of the Prairies, or investing in a processing plant themselves. Given that sea buckthorn products are a generally unknown commodity in North America it is difficult to assess demand, therefore considerable market research and industry development is required. Partnerships with government, industry, bioproduct researchers and producers need to be established to fully investigate opportunities for sea buckthorn.

There is little publicly available information on sea buckthorn processing. This information is important to potential investors in the industry and in particular producers who are considering integrating forward into primary and/or secondary processing. This information is also important to assessing individual and industry viability. The analysis should include the alternative methods of processing such as CO_2 or hexane extraction as well as cold pressing technologies.

An organization of sea buckthorn producers will be important to the future development of the sea buckthorn industry in Saskatchewan. Organized producers have the ability gather support for research and development of the industry in a more effective manner than individual producers or processors. They can also can provide information to new growers and act as an industry development vehicle. This is the first step to the eventual formation of a Sea Buckhtorn Council should the sea buckthorn prove to have the economic potential for full industry development.

It is a pioneer species, and often the first woody species colonizing open areas such as abandoned farmland, wasteland, and rocky islands. It has numerous stems and will form a colony if left to grow naturally and spread by rhizomes. Seedlings begin to form suckers after as little as three years growth, with the suckers then producing their own roots. The root system of sea buckthorn may have a taproot or it may have irregularly spaced taproots. Like other members of the *Elaeagnaceae* family (buffaloberry, Russian olive) sea buckthorn is a nitrogen fixer. This means that sea buckthorn is able to assimilate atmospheric nitrogen, and has the potential to contribute significant amounts of nitrogen to the soil, probably as much as legumes and more than many actinorhizal plants.

Observations during course of the project suggest that sea buckthorn is one of the hardiest and most adaptable woody plants used in prairie conservation programs. In these plantings, sea buckthorn forms shrubs or small trees and appears to have synergistic effect on understory vegetation. Thus sea buckthorn appears to be a near-ideal candidate for agroforestry development in Saskatchewan on the prairies. Integrating this high value plant into crop and livestock production systems may offer several environmental benefits, such as farmstead protection, erosion control, riparian protection, mine land reclamation, and enhancement of wildlife habitats.

Agricultural intensification in Saskatchewan has altered natural ecosystems and has resulted in the degradation of the natural habitats of numerous native plants, animals and microorganisms. Through its synergy effects, sea buckthorn is expected to alleviate many of the environmental problems in Saskatchewan agroecosystems.

Along with its economic benefits, the environmental benefits of sea buckthorn need to be rigorously evaluated in two major agroforestry plantations, hedgerow intercropping and silvopastoral systems, especially in marginal farmlands in Saskatchewan.

Appendix 1: Hippophae Species and Subspecies Represented in the Project						
Species	**Subspecies**	**Accession**	**Source**	**ID/Name**	**Form**	**Originator**
goniocarpa		E6561	Summerland		OPS	AAFC-RES
		EP6467	China	HGO-1	OPS	UofS
neurocarpa		EP6469	China	HN-2	OPS	UofS
		EP6470	China	HN-1	OPS	UofS
		EP6474	China	HN-3	OPS	UofS
gyantensis		E6562	Summerland		OPS	AAFC-RES
		E6563	Summerland		OPS	AAFC-RES
		EP6468	China	HG-1	OPS	UofS
rhamnoides	Mongolica		Indian Head	Indian Summer	OPS	AAFC-PFRA
	"		Fillmore	S1	Veg	AAFC-PFRA
	"		Fillmore	S2	Veg	AAFC-PFRA
	"		Fillmore	S3	Veg	AAFC-PFRA
	"		Fillmore	S4	Veg	AAFC-PFRA
	"		Fillmore	S5	Veg	AAFC-PFRA
	"		Dafoe	TB-1	Veg	AAFC-PFRA
	"		Dafoe	TB-2	Veg	AAFC-PFRA
	"		Dafoe	TB-3	Veg	AAFC-PFRA
	"		Dafoe	TB-4	Veg	AAFC-PFRA
	"		Dafoe	TB-5	Veg	AAFC-PFRA
	"		Dafoe	TB-6	Veg	AAFC-PFRA
	"		Dafoe	TB-7	Veg	AAFC-PFRA
	"		Dafoe	TC-3	Veg	AAFC-PFRA
	"		Bacarres	Bal-1	Veg	AAFC-PFRA
	"		Bacarres	Bal-7	Veg	AAFC-PFRA
	"		Bacarres	Bal-C4	Veg	AAFC-PFRA
	"		Francis	Fr-13	Veg	AAFC-PFRA
	"		Francis	Fr-14	Veg	AAFC-PFRA
	"		Hudson Bay	HB-1	Veg	AAFC-PFRA
	"		Melville	M-1	Veg	AAFC-PFRA
	"		Melville	M-2	Veg	AAFC-PFRA
	"		Melville	M-3	Veg	AAFC-PFRA
	"		Melville	M-5	Veg	AAFC-PFRA
	"		Melville	M-7	Veg	AAFC-PFRA
	"		Melville	M-10	Veg	AAFC-PFRA
	"		Melville	M-13	Veg	AAFC-PFRA
	"		Melville	M-14	Veg	AAFC-PFRA
	"		Melville	M-16	Veg	AAFC-PFRA
	"		Melville	M-19	Veg	AAFC-PFRA
	"		Melville	M-22	Veg	AAFC-PFRA
	"		Outlook	OL-1	Veg	AAFC-PFRA
	"		Outlook	OL-2	Veg	AAFC-PFRA
	"		Outlook	OL-6	Veg	AAFC-PFRA
	"		Regina	WC-S2	Veg	AAFC-PFRA
	"		Regina	WC-S3	Veg	AAFC-PFRA
	"		Regina	WC-S4	Veg	AAFC-PFRA
	"		Rafferty	R-38	Veg	AAFC-PFRA
	"		Rafferty	R-41	Veg	AAFC-PFRA
	"		Rafferty	R-43	Veg	AAFC-PFRA

	"		Rafferty	R-L	Veg	AAFC-PFRA
	"		Rafferty	R-C4	Veg	AAFC-PFRA
	"	E6559	Summerland		OPS	AAFC-RES
	"	EP6476	Russia	SB-F-1	OPS	UofS
	"		Russia	6476-2	Veg	UofS
	"		SBC	6582	Veg	AAFC-PFRA
	"		SBC	6583	Veg	AAFC-PFRA
	"		SBC	6584	Veg	AAFC-PFRA
	"		SBC	6581	Veg	AAFC-PFRA
	"		Russia	Alei	Veg	Novosibirsk
	"		Russia	Orangenaya	Veg	Barnaul, Vavilov
	"		Russia	Obilnaya	Veg	Barnaul, Vavilov
	"		Russia	Yantarnaya	Veg	Barnaul
	"		Russia	Vitaminaya	Veg	Barnaul, Vavilov
	"		Russia	Zivko	Veg	Novosibirsk
	"		Russia	Chechek	Veg	Novosibirsk
	"		Russia	Panteleevskaya	Veg	Novosibirsk
	"		Russia	Prevoskhodnaya	Veg	Vavilov, Lebedev
	"		Russia	Dar Kartuni	Veg	Vavilov
	"		Russia	Podorok Sadu	Veg	Vavilov
	"		Russia	Limarnaya	Veg	Vavilov
	"		Russia	Trofimovskaya	Veg	Vavilov
	"		Russia	Samorodok	Veg	Lebedev
	"		Russia	Rodnotchok	Veg	Lebedev
	"		Russia	17-65	Veg	Lebedev
	"		Russia	Velikan	Veg	Lebedev
	"	EP5789	China		OPS	McLoughlin
	"	EP6357	China		OPS	McLoughlin
rhamnoides	rhamnoides	E4715	Volgograd		OPS	Mattis
	"		Latvia	Proznachnaya	Veg	Bruvellis
	"		Latvia	Ljubitelskaya	Veg	Bruvellis
	"	E6566	Summerland		OPS	AAFC-RES
	"		Germany	Leikora	Veg	Albrecht
	"		Germany	Frugana	Veg	Albrecht
	"		Germany	Hergo	Veg	Albrecht
	"		Germany	Pollmix 1	Veg	Albrecht
	"		Germany	Pollmix 2	Veg	Albrecht
	"	EP6477	Finland	A-F	OPS	UofS
	"	EP6478	Finland	AF 30/5	OPS	UofS
	"	EP6479	Canada	SAS-1	OPS	UofS
	"	EP6480	Finland	17-R	OPS	UofS
	"	EP6481	Finland	F-15	OPS	UofS
	"	EP6482	Finland	F-14	OPS	UofS
	"	EP6483	Finland	A-17	OPS	UofS
	"	EP6484	Finland	F-17	OPS	UofS
	"	EP6485	Finland	F-11	OPS	UofS
	"	EP6359	China		OPS	McLoughlin
rhamnoides	sinensis	EP6361	China		OPS	McLoughlin
	"	E6560	Summerland		OPS	AAFC-RES
	"	EP6455	China	C-5	OPS	UofS
	"	EP6456	China	C-18	OPS	UofS
	"	EP6457	China	C-13	OPS	UofS
	"	EP6458	China	C-14	OPS	UofS

	"	EP6459	China	C-10	OPS	UofS
	"	EP6460	China	C-8	OPS	UofS
	"	EP6461	China	C-3	OPS	UofS
	"	EP6462	China	C-6	OPS	UofS
	"	EP6463	China	AC	OPS	UofS
	"	EP6534	China	C-16	OPS	UofS
	"	EP6535	China	POP-17	OPS	UofS
rhamnoides	turkestanica	EP6464	China	C-2	OPS	UofS
	"	EP6465	China	C-1	OPS	UofS
	"	EP6466	China	AC-1	OPS	UofS
	"	E6558	Summerland		OPS	AAFC-RES
salicifolia		E6564	Summerland		OPS	AAFC-RES
		EP6360	China		OPS	UofS
		EP6454	China	HS-1	OPS	UofS
tibetana		E6565	Summerland		OPS	AAFC-RES
		EP6358	China		OPS	McLoughlin
		EP6471	China	HT-3	OPS	UofS
		EP6472	China	HT-2	OPS	UofS
		EP6473	China	HT-1	OPS	UofS
		EP6486	China	HT-4	OPS	UofS

OPS = Open pollinated seedling
Veg = Vegetatively propagated

Appendix Table 2: Characteristics of sea buckthorn selections							
Location	Selection	Fruit Wt g/100	Pedicel Length (mm)	Fruit Removal Force (g)	Harvest Index	Seed Oil (%)	Seed Wt g/100
Balcarres	BAL-7	53.2	6.6	114	3.1	16.2	2.0
Dafoe	TB-1	31.2	2.8	138	0.6	13.5	1.4
Dafoe	TB-3	28.4	3.6	135	0.8	15.3	1.5
Dafoe	TB-5	30.6	2.2	150	0.4	13.0	1.6
Dafoe	TB-10	40.4	4.0	141	1.1	14.5	1.8
Francis	FR-4	43.9	4.1	160	1.1	14.7	1.9
Francis	FR-11	42.8	3.8	152	1.1	16.1	1.8
Francis	FR-13	43.2	4.3	101	1.8	14.6	2.0
Francis	FR-14	65.1	7.8	136	3.7	13.9	
SBC	H6-2	40.6	3.1	31	4.0	14.3	1.6
SBC	H6-5A	40.3	3.6	118	1.2	16.2	2.0
SBC	H6-21	34.6	3.5	100	1.2	15.3	
SBC	H6-32	33.4	3.5	135	0.9	15.8	1.5
SBC	H6-34	40.9	3.8	69	2.2	14.6	1.7
SBC	H6 - 50	38.6	5.9	95	2.4		1.6
SBC	6582	84.2	4.6	69	5.6	11.1	2.2
SBC	6583	45.3	6.2	99	2.8		
SBC	6584	60.0	3.4	100	2.0		
SBC	6476-2	67.5	3.9	75	3.5	12.2	2.1
Summerberry	SB-1	43.3	2.2	79	1.2	10.8	1.6
Summerberry	SB-5	52.1	3.4	150	1.2	14.2	2.1
Summerberry	SB-20	53.6	4.0	165	1.3	10.9	1.8
Summerberry	SB-50	41.6	3.7	133	1.2	14.5	1.8
Summerberry	SB-25	57.2	4.4	221	1.1	11.5	2.1
Outlook	OL-1	47.8	2.9	138	1.0	14.9	2.0
Outlook	OL-6	53.9	4.0	112	1.9	13.8	2.1
Outlook	OL - 2	33.1	4.1	109	1.3		1.8
Odessa	OD-2	42.1	4.8	131	1.5	13.0	1.4
Odessa	OD-12	44.3	3.1	165	0.8	12.5	2.3
Odessa	OD-14	50.6	3.5	151	1.2		1.9
Odessa	OD-15	40.5	4.2	132	1.3	14.5	1.8
Odessa	OD-16	38.2	3.0	112	1.0	14.4	1.7
Odessa	OD-17	52.5	4.9	145	1.8	15.7	1.8
Rafferty	R-38	72.6	4.9	145	2.4	14.7	1.9
Rafferty	R-41	66.0	5.3	116	3.0	14.2	1.8
Rafferty	R-L	52.4	5.7	132	2.3	17.1	2
Rafferty	R-C4	45.4	6.9	105	3.0	17.4	2.2
Rafferty	R-43	48.9	5.7	104	2.7	15.1	2.1
Wascana	WC - S1	47.5	3.9	124	1.5	16.3	2.1
Wascana	WC - S2	32.1	3.4	141	0.8	16.0	1.9
Wascana	WC - S3	45.9	5.4	103	2.4	16.2	2.2
Wascana	WC - S4	33.2	3.7	100	1.2	16.1	1.7
Melville	M-1	44.5	3.1	177	0.8	14.9	1.9
Melville	M-22	44.1	5.3	112	2.1	15.3	2.0
Melville	M-7	42.2	5.4	136	1.7	15.4	2.2

Appendix Table 2A: Top Female Seabuckthorn Selections - 1999						
Location #	Location Name	Selection	Fruit Wt g/100 Berries	Fruit (n=10) Pedicel Length (mm)	Fruit Removal Force (g)	Seed Wt g/100 seed
1	Balcarres	Bal - 1	48.08	4.41	157.4	1.80
1	Balcarres	Bal - 7	53.15	6.59	114.3	1.97
2	Dafoe	TB - 2	54.65	3.54	137.68	1.93
3	Fillmore	S - 5	52.10	3.39	127.47	2.08
4	Francis	Fr - 14	65.11	7.75	136.3	2.07
6	Melville	M - 1	44.52	3.06	176.6	2.10
6	Melville	M - 7	42.24	5.37	136.13	1.91
6	Melville	M - 10	39.18	5.26	125.03	2.00
6	Melville	M - 13B	53.90	3.59	159.55	1.70
6	Melville	M - 14	54.64	5.14	123.3	1.94
6	Melville	M - 16	41.27	4.40	106.87	1.42
6	Melville	M - 22	44.06	5.26	111.57	1.50
7	Outlook	OL - 1	47.80	2.94	138.13	2.03
7	Outlook	OL - 2	33.12	4.12	108.87	1.82
7	Outlook	OL - 6	53.90	3.96	112.37	2.08
8	Rafferty	R - 38	72.63	4.85	144.63	1.91
8	Rafferty	R - 41	66.01	5.27	115.9	1.83
8	Rafferty	R - 43	48.85	5.69	103.53	2.08
8	Rafferty	R - L	52.42	5.73	131.5	1.98
8	Rafferty	R - C4	45.42	6.91	104.7	2.20
9	SBC	H6 - 5A	40.29	3.59	117.5	2.04
9	SBC	H6 - 35	55.67	4.48	105.97	1.78
9	SBC	H6 - 50	38.60	5.86	94.87	1.61
9	SBC	N4 - 17	46.34	4.02	138.23	2.03
9	SBC	N4 - 19	51.81	4.98	137.48	2.20
10	Summerberry	SB - 25	57.20	4.42	221.3	2.10
10	Summerberry	SB - 26	46.16	4.02	133.2	1.66

Appendix Table 3: Fatty Acid Composition of Sea Buckthorn Selections							
	% Fatty Acid						
Selection	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Others*	Total
BAL-7	6.83	3.02	17.33	35.18	35.65	1.99	100.00
FR-11	7.14	3.52	19.76	34.21	33.45	1.93	100.00
FR-14	6.10	2.86	15.30	34.69	39.31	1.74	100.00
H6-5A	6.91	2.89	21.32	28.17	38.77	1.94	100.00
M-14	6.62	3.17	15.95	35.91	36.49	1.86	100.00
M-7	6.81	2.92	16.26	32.14	39.92	1.96	100.00
N4-19	6.69	2.83	14.51	34.32	39.76	1.89	100.00
Od-12	6.53	3.44	23.51	31.15	33.71	1.66	100.00
OL-6	6.09	2.79	14.07	36.14	39.22	1.71	100.00
R-15	6.00	3.75	22.64	32.30	33.74	1.58	100.00
R-38	6.46	3.60	19.64	32.97	35.46	1.87	100.00
R-39	6.74	3.23	16.82	38.05	33.47	1.70	100.00
R-43	6.08	3.43	19.42	35.51	33.73	1.84	100.00
R-46	5.91	3.38	20.28	33.52	35.22	1.70	100.00
R-C-4	6.41	2.90	18.67	34.00	36.25	1.76	100.00
R-L	6.65	3.18	17.11	33.87	37.30	1.89	100.00
SB-25	6.01	2.79	16.67	31.79	40.84	1.89	100.00
WC-S1	7.64	3.10	15.38	34.23	37.83	1.82	100.00
WC-S2	6.38	3.61	17.73	34.15	36.37	1.75	100.00
WC-S3	6.34	3.04	16.84	39.82	32.38	1.59	100.00
WC-S4	6.46	3.55	16.81	34.59	36.85	1.73	100.00

Appendix Table 4: Fatty acid composition of oil from sea buckthorn Selections in Francis and Dafoe									
			% Fatty Acid						
Site	Tree	Year	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Others	Total
Francis	FR-4	1997	7.18	2.78	15.62	38.01	34.36	2.05	100.0
Francis	FR-4	1998	6.38	2.73	15.70	34.89	38.34	1.97	100.0
Francis	FR-4	1999	6.28	2.81	14.66	36.51	37.90	1.85	100.0
Francis	FR-6	1997	6.56	2.96	21.31	36.21	31.19	1.78	100.0
Francis	FR-6	1998	6.07	3.32	23.13	33.01	32.72	1.75	100.0
Francis	FR-6	1999	5.97	2.92	21.86	33.07	34.57	1.61	100.0
Francis	FR-13	1997	6.67	2.98	16.68	39.60	32.24	1.84	100.0
Francis	FR-13	1998	5.86	3.03	16.07	37.77	35.41	1.86	100.0
Francis	FR-13	1999	5.72	3.31	16.50	36.91	35.83	1.74	100.0
Dafoe	TB-1	1997	6.63	2.59	15.49	32.13	41.35	1.81	100.0
Dafoe	TB-1	1998	6.63	2.76	14.65	33.96	40.14	1.86	100.0
Dafoe	TB-1	1999	6.77	2.87	14.92	32.38	41.21	1.85	100.0
Dafoe	TB-2	1997	6.36	2.87	28.66	32.69	27.37	2.05	100.0
Dafoe	TB-2	1998	6.38	3.32	23.18	31.50	33.77	1.85	100.0
Dafoe	TB-2	1999	5.96	3.20	19.12	33.95	36.01	1.76	100.0
Dafoe	TB-5	1997	7.33	2.88	15.01	38.13	34.50	2.12	100.0
Dafoe	TB-5	1998	6.62	3.11	17.36	33.20	37.65	2.05	100.0
Dafoe	TB-5	1999	6.82	3.12	13.48	34.89	39.46	2.23	100.0

Appendix Table 5: Seed Oil Content and Seed Weight of Sea Suckthorn Selections

Selection	Oil Content (%)	Seed Weight (g/100 seeds)
R-46-98	17.79	1.81
R-C-4-99	17.78	2.20
H6-36-97	17.51	1.50
R-39-98	17.48	1.96
R-15-98	17.45	1.89
SB-22-97	17.13	1.61
R-L-98	17.07	1.81
H6-32-97	16.97	1.53
R-L-99	16.97	1.98
H6-5A-99	16.85	2.04
Fr-6-97	16.70	1.54
SB-23-97	16.66	1.57
Bal-7-97	16.55	
T-3-99	16.48	
Fr-11-99	16.47	1.82
R-43-99	16.47	2.08
SB-42-97	16.44	1.65
Fr-10-97	16.40	1.54
TB-2-99	16.40	1.93
Bal-7-98	16.39	1.95
TB-2-97	16.39	1.74
H6-50-99	16.36	1.61
Od-12-99	16.34	2.26
R-T-98	16.33	1.65
R-11-98	16.32	1.69
Wc-S1-99	16.28	2.08
Wc-S3-99	16.16	2.23
Fr-11-97	16.14	1.70
M-10-99	16.13	2.00
Wc-S4-99	16.11	1.7
H6-2-97	16.10	1.93
SB-26-99	16.06	
M-14-99	16.02	1.94
R-C-4-98	16.00	1.66
Fr-10-98	15.99	1.60
Fr-6-98	15.96	1.86
Wc-S2-99	15.96	1.89
M-7-99	15.92	1.91
N4-16-98	15.92	1.83
M-13-99	15.90	
R-D-98	15.90	1.93
M-9-98	15.87	1.75
SB-7-97	15.87	1.97
R-24-98	15.86	1.95
R-12-98	15.84	1.56
Fr-12-98	15.82	1.86
H6-21-97	15.82	1.80
Bal-7-99	15.80	1.97
H6-1A-98	15.74	1.88
R-47-98	15.74	1.66
M-16-98	15.71	2.10
Fr-4-98	15.70	1.92
Od-17-99	15.69	1.82
Fr-13-99	15.68	2.04
H6-5A-98	15.66	2.32
R-38-99	15.63	1.91
SB-26-98	15.63	1.86
Ol-2-99	15.56	
M-11-98	15.54	1.69
M-4-98	15.53	1.59
Ol-1-99	15.53	2.03
R-34-98	15.53	1.63
M-17-98	15.50	1.65

M-22-99	15.50	1.50
R-33-98	15.49	1.81
Fr-11-98	15.46	1.98
M-6A-98	15.46	1.91
TB-2-98	15.45	1.92
H6-21-99	15.38	
TB-3-98	15.35	1.46
R-19-98	15.31	1.43
M-10-98	15.28	2.00
H6-32-98	15.27	1.52
N4-11-98	15.27	2.31
HB-5A-98	15.25	
H6-32-99	15.23	1.46
M-1-99	15.22	2.10
Fr-13-97	15.18	2.03
M-13-98	15.17	1.65
M-21-98	15.17	1.69
R-37-98	15.17	1.46
R-8-98	15.14	1.62
R-40-98	15.13	1.74
M-22-98	15.12	1.59
Ol-1-97	15.12	
SB-50-97	15.12	1.92
Fr-7-98	15.11	1.51
SB-50-98	15.11	1.80
HB-3-97	15.10	
H6-5-98	15.08	1.52
Fr-4-99	15.07	1.86
R-28-98	15.07	1.24
R-25-98	15.06	1.50
H6-34-97	15.05	1.93
M-2-98	14.99	1.52
HB-1-97	14.97	
R-14-98	14.97	1.49
TB-10-97	14.96	1.63
M-8-98	14.94	1.32
S-5-99	14.94	2.08
Ol-6-99	14.90	2.08
H6-35-99	14.88	1.78
M-7-98	14.87	1.80
Bal-4-97	14.86	
R-9-98	14.85	1.63
R-35-98	14.83	1.38
R-30-98	14.80	1.63
HB-5B-98	14.79	
N4-19-99	14.78	2.20
Bal-3-98	14.75	1.56
V6-1-97	14.75	
Fr-1-98	14.73	1.50
H6-34-98	14.73	1.71
S-3-98	14.72	1.40
SB-14-97	14.70	1.76
H6-21-98	14.68	1.96
SB-33-98	14.68	1.70
N4-40-98	14.66	1.75
N4-A3-98	14.65	1.73
R-13-98	14.62	1.53
H6-31-A-97	14.58	
M-1-98	14.58	2.02
Fls-10-98	14.56	
Fr-6-99	14.56	1.75
M-16-99	14.55	1.42
SB-56-98	14.52	1.80
TB-1-99	14.52	1.4
HB-1-98	14.51	1.37
R-10-98	14.51	1.37
Fr-3-98	14.48	1.67
R-G-98	14.48	1.67
R-41-99	14.44	1.83
M-18-98	14.43	1.47

Od-16-99	14.43	1.72
SB-10-97	14.43	1.79
H6-50-98	14.42	1.60
Fls-15-98	14.40	
HB-4-98	14.39	
S-6-98	14.37	1.29
HB-2-97	14.36	
N4-12-98	14.34	1.45
M-5-98	14.32	1.58
OL-4-98	14.32	1.93
R-16-98	14.32	1.70
H6-31-B-97	14.31	
R-44-98	14.31	1.70
TB-10-98	14.29	1.83
Bal-1-98	14.28	1.59
M-19-98	14.25	1.54
TB-10-99	14.25	1.79
Fr-10-99	14.21	1.53
R-32-98	14.20	1.83
H6-34-99	14.19	1.67
M-12-98	14.19	1.65
SB-41-98	14.19	1.57
N4-19-98	14.18	2.11
SB-21-98	14.14	1.72
Bal-5-98	14.12	1.86
SB-20-97	14.12	1.71
R-41-98	14.07	1.29
Fr-5-98	14.06	1.91
H6-2-98	14.04	1.63
OL-1-98	14.04	1.82
TB-4-98	14.04	1.30
M-3-98	14.02	1.80
R-P-98	14.02	1.57
H6-1-98	14.01	1.67
Fr-14-99	13.99	2.07
SB-50-99	13.99	1.77
H6-33-98	13.97	1.53
SB-31D-98	13.94	1.75
M-14-98	13.93	1.79
SB-31C-98	13.93	1.62
SB-57-98	13.91	1.90
Od-C-2-99	13.89	
R-18-98	13.86	1.69
SB-30-98	13.86	1.59
Bal-4-98	13.85	1.69
Fr-14-98	13.83	1.75
N4-34-98	13.83	1.85
R-45-98	13.83	1.28
M-15-98	13.81	1.55
R-38-98	13.81	2.04
SB-3-97	13.79	1.76
H6-3-98	13.78	1.54
OL-8-98	13.78	1.97
Bal-6-98	13.77	1.56
SB-5-98	13.76	1.75
OL-2-98	13.74	2.10
R-43-98	13.73	1.66
H6-7B-98	13.71	1.49
R-42-98	13.71	1.77
Fr-2-98	13.70	1.73
SB-24-98	13.70	1.55
R-31-98	13.68	1.42
Fr-9-98	13.67	2.04
M-20-98	13.65	1.57
TB-7-97	13.60	1.63
Od-14-99	13.56	1.88
R-C-98	13.56	
SB-1-97	13.56	1.90
HB-2-98	13.55	1.78
S-5-98	13.54	1.96

H6-35-98	13.51	1.65
SB-40-98	13.51	1.72
N4-31-98	13.50	
SB-55-98	13.50	1.84
R-K-98	13.36	1.38
H6-3B-98	13.35	1.66
Od-1-97	13.33	
SB-48A-98	13.30	
TB-1-98	13.30	1.59
R-Q-98	13.29	1.42
Fr-4-97	13.26	1.90
TB-5-99	13.24	1.64
OL-3-98	13.21	1.63
OL-5-98	13.13	1.80
SB-28-98	13.12	1.53
TB-5-98	13.08	1.90
R-48-98	13.06	1.41
N4-17-98	13.05	1.88
R-M-98	13.04	1.29
0d-2-99	13.03	
R-36-98	13.03	1.53
Fr-13-98	12.98	1.97
SB-27-98	12.92	1.70
Fr-3-99	12.90	1.63
M-6B-98	12.90	1.63
TB-1-97	12.85	
OL-7-98	12.79	1.83
Fr-7-97	12.77	
R-I-98	12.75	1.44
R-R-98	12.75	1.49
OL-6-98	12.74	2.00
H6-2-99	12.72	1.62
N4-37-98	12.67	1.36
TB-5-97	12.67	1.67
H6-4-98	12.64	1.71
SB-46-98	12.51	1.84
Od-15-99	12.49	1.77
SB-47A-98	12.45	1.76
SB-25-98	12.41	2.16
SB-48-98	12.36	
R-7-98	12.30	1.70
N4-22-98	12.23	1.79
SB-49-98	11.97	1.53
SB-25B-98	11.95	1.60
SB-4-98	11.88	1.80
SB-45-98	11.77	2.00
N4-17-99	11.65	2.03
N4-41-98	11.63	1.63
R-17-98	11.59	1.40
HB-3-98	11.43	1.49
R-J-98	11.33	1.87
SB-35-98	11.18	1.78
SB-25-99	11.11	2.10
N4-32-98	10.78	1.29
SB-20-98	10.57	1.82
SB-43-98	10.45	1.37
SB-1-99	9.88	1.6
SB-34-98	9.29	1.56
S-4-98	9.02	1.40
SB-1-98	8.91	1.72
V-2-98	7.87	
SB-20-99	7.86	1.84
Bal-1-99	7.71	

Addendum to ADF Final Report

Project # 98000273

Title : Sea Buckthorn Cultivar and Orchard Development in Saskatchewan

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Summary:

Thirty-two accessions of Sea buckthorn (*Hippophae* spp.) from the Sea Buckthorn Germplasm Nursery maintained at PFRA, Indian Head, Saskatchewan (SK) were analyzed for genetic diversity using the amplified fragment length polymorphism (AFLP) technique. Twelve AFLP primers were used to selectively amplify DNA from restricted samples of 32 accessions, and 7 clones of these accessions. Amplification patterns of 4 primer pairs were analyzed using the NTSys Biostatistical software for similarity. The results of these analyses indicated that all samples within the collection showed at least a 58% similarity. In addition, a couple of distinct geographically grouped linkages could be discerned. The majority of accessions collected from Saskatchewan were clustered with a few accessions from China and Finland. A close clustering of some accessions from China was noted. No other distinct geographically based clusters were observed. The genetic diversity of five accessions developed as commercial cultivars during the project was also examined. Three of the accessions (RC-4, FR-14, and EP6482) clustered together with two additional accessions from Saskatchewan (Bal-7 and N4-19). The other two accessions (OL-6 and EP6476) were found to cluster with two accessions collected from Rafferty SK. Four clones from two accessions were analyzed to verify genetic stability. In both sets of clones no significant difference in the amplification patterns of DNA from the clones could be detected.

Introduction and Objective

Amplified fragment length polymorphism (AFLP) is a genomic DNA fingerprinting method used for taxonomic distinction between individuals at the species or subspecies level. It reveals genetic variation through polymorphism between genomic fragments that have been amplified. he fragments amplified are the restricted DNA fragments that have been digested by both enzymes used in the procedure. The variation in the amplification of the products is based on the selective primers that are used in the PCR reactions. As the genetic variation that exists between any two samples increases, the number of amplification products that differ between the two species will increase. A statistical analysis of the amplification pattern can then be used to compare the relatedness between two or more individual samples.

The above approach can be used to determine the relatedness of any two individuals, to determine if two individuals are genetically identical (clones) and to determine the genetic diversity within populations. The latter point is of paramount importance. Genetic variation within a population will improve the likelihood of successful exploitation of an ecosystem, as the population will contain increased genetic resources to adapt to changes and challenges of new and changing environment.

The purpose of this extension was to use AFLP analysis to determine the genetic diversity that exists among the sea buckthorn accessions assembled during the project period and maintained at PFRA, Indian Head, and determine whether adequate genetic diversity exists among cultivars being developed for release.

Materials and Methods

Plant material: Leaf tissue from 28 accessions of sea buckthorn (Table 1) grown at the Germplasm Nursery at PFRA, Indian Head SK, was frozen in liquid nitrogen and then stored at -80°C until extraction. After extraction of the DNA from these samples, it was noted that 4 of the accessions that had been developed for release in Saskatchewan had not been harvested. Dormant plants of these accessions were subsequently obtained for the study. The plants were placed at 22°C under16 h photoperiod to break dormancy. Leaf tissue was collected after three weeks of growing under inductive conditions.

DNA Extraction: DNA was extracted using the Qiagen Plant Dneasy Maxi Kit. Approximately 1 g of frozen tissue was ground in mortar and pestle with liquid nitrogen. The remainder of the extraction protocol was according to manufacturer's instructions. DNA was quantified using UV absorption at 260nm and by visual inspection after staining with ethidium bromide. DNA was then diluted to a concentration of 100 ng/l. with sterile ddH_2O.

AFLP Analysis: AFLP analysis was initiated using both the large and small genome AFLP kits (Invitrogen Ltd). Following this initial trial, it was decided to utilize the large genome kit for the bulk of the samples for superior results. Thirty-nine samples were processed using the large genome kit (32 accessions plus an additional 6 clones (3 each from 2 accession)). The 39^{th} sample was one of the original 32 that was extracted twice and used as an internal control to test the reproducibiltiy of the AFLP reaction. Reactions were completed as per instructions provided with the kit, using 250 ng of DNA per reaction. In total, 12 primers (6 pairs) were used. The reactions were analyzed using polyacrylamide gel electrophoresis and silver staining.

Gel electrophoresis and staining: A 6 % urea:polyacrylamide gel was used to separate the AFLP reaction products. The samples were run at 60W for 3 hours using a BioRad Sequencing vertical electrophoresis apparatus. Following the electrophoreses, the plates were separated, and the gel was stained as follows:

The gel was fixed by placing in 10 % glacial acetic acid for 20 minutes, washed three times with ddH_2O and placed in staining solution (1 gAgNO3 per l of 0.015% formaldehyde) for 30 minutes. The gel was then rinsed for 10 seconds in ddH_2O and placed in chilled developing solution (30g sodium carbonate, 1.5 ml 37% formaldehyde and 2.5 mg of sodium thiosulphate per liter). The gel was left in the developing solution until bands could be visualized (2 – 5 minutes). Once a suitable band intensity was developed, the reaction was stopped by adding an equal volume of 10% acetic acid to the solution for10 minutes. The gel was then removed and allowed to air-dry overnight.

Statistical Analysis: After drying, the gels were scored for polymorphic bands that were clearly distinguishable on the gels. The result of these analyses were entered into an Excel spreadsheet using the code 1 if a band was present, 0 if absent and 9 if the reaction did not work or was unclear in that sample. Using this criterion, 58 polymorphic bands were entered into the spreadsheet. The spreadsheet was then imported into the

biostatistical software program NTSys Version 2.1 (Exeter Software). Qualitative similarity analyses were run using three coefficients, DICE, SM and J. The results were then clustered to create the dendrograms presented in figures 1 – 3.

Table 1 List of Sea buckthorn accessions included in the DNA assay. All accessions were collected from PFRA, Indian Head, SK.

Sample	Accession Name	Geographical Source	*Hippophae* Species
1	EP6463	China	*rhamnoides*
2	EP6478	Finland	*rhamnoides*
3	EP6480	Finland	*rhamnoides*
4	EP6485	Finland	*rhamnoides*
5	EP6456	China	*rhamnoides*
6	EP6469	China	*neurocarpa*
7	EP6457	China	*rhamnoides*
8	EP6459	China	*rhamnoides*
9	EP6465	China	*rhamnoides*
10	EP6476**	Russia	*rhamnoides*
11	OL-1	Outlook	*rhamnoides*
12	R-41	Rafferty	*rhamnoides*
13	N4-11	SBC	*rhamnoides*
14	M6-126	Melville	*rhamnoides*
15	EP6477	Finland	*rhamnoides*
16	EP6458	China	*rhamnoides*
17	M-19	Melville	*rhamnoides*
18	TB2E-6595	Dafoe	*rhamnoides*
19	EP6463	China	*rhamnoides*
20	EP6468	China	*rhamnoides*
21	EP6479	Canada	*rhamnoides*
22	EP6483	Finland	*rhamnoides*
23	WC-S4	Regina	*rhamnoides*
24	FR-13	Francis	*rhamnoides*
25	WC-S3	Regina	*rhamnoides*
26	EP6455	China	*rhamnoides*
27	N4-19	SBC	*rhamnoides*
28	BAL-7	Balcarres	*rhamnoides*
29	R38	Rafferty	*rhamnoides*
30	OL-6**	Outlook	*rhamnoides*
31	FR-14**	Francis	*rhamnoides*
32	RC-4**	Rafferty	*rhamnoides*
33	EP6582**	SBC	*rhamnoides*

Accessions marked with ** are those developed for cultivar release during the project. SBC denotes sea buckthorn cultivar.

Results:

Three questions were addressed in this extended part of the project: (a) were the clones genetic stable and identical, (b) what was the genetic relatedness of the collection of sea buckthorn conserved at PFRA, and (c) was there sufficient genetic diversity in the accessions developed for release as cultivars (SBC) during the project? Six selective primer sets were used and four of these sets were analyzed. In total 58 polymorphic bands were scored for statistical treatment.

Identity of clones: Four clones of the accessions EP6457 and EP6463 were analyzed (data not presented). No major polymorphoric differences could be observed in the clones of either accession. Time and logistics dictated only assessing the identity of two accessions that were selected at random. Also these samples were only analyzed with a single primer pair. Although the scope of the experiment was limited the results would indicate that there is a very high probably of maintaining genetic stability through this form of propagation.

Genetic relatedness of accessions: The three dendrograms represent an analysis of the AFLP polymorphism detected using the four primer pairs (Figures 1, 2 and 3).

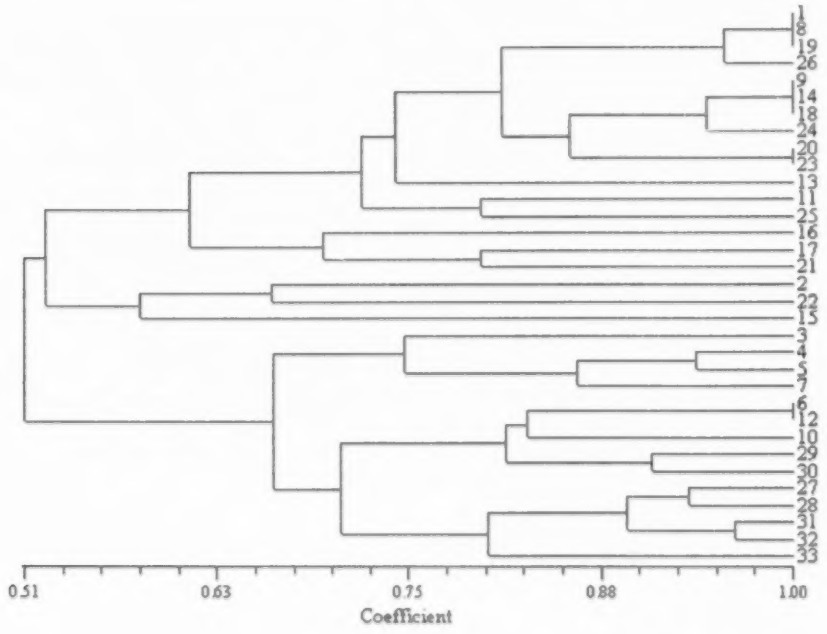

Figure 1: Dendrogram of sea buckthorn accession as generated from AFLP analysis using the DICE coefficient

It should be noted that although 58 polymorphic bands were scored in total, not all accessions were scored for all 58 bands. A number of DNA samples did not work in all

reactions, and in many cases, it was unclear if a band was present or absent in a given sample (see Figure 4). However through the use of the four different primer pairs, all DNA samples were successfully amplified with at least two primer pairs.

Clearly all three dendrograms display a great deal of similarity in calculating the relatedness. A couple of significant geographical groupings of accessions were observed with several samples obtained from China (Accessions 1 and 19, and 8 and 26) clearly clustering together. Accessions from Finland (2, 3, 4, 15, and 22) grouped as a unit, with the tightest grouping shown between accessions 15 and 22. The exception to the above is observed when the DICE coefficient (Figure 1) is used and the cluster is expanded to include two additional Finnish accessions (2 and 3). A number of the accessions collected from various locations throughout Saskatchewan also display a substantial degree of relatedness. Accessions 3, 9,13, 14, 18, 23, 24, and 25 grouped within a short genetic distance and was best displayed using the SM coefficient (Figure3). However, there was still considerable genetic diversity within the collection of sea buckthorn in the province. There was no clear genetic bias to either the Chinese or Finland accessions within the Saskatchewan collection as different clusters included accessions from one or the other country.

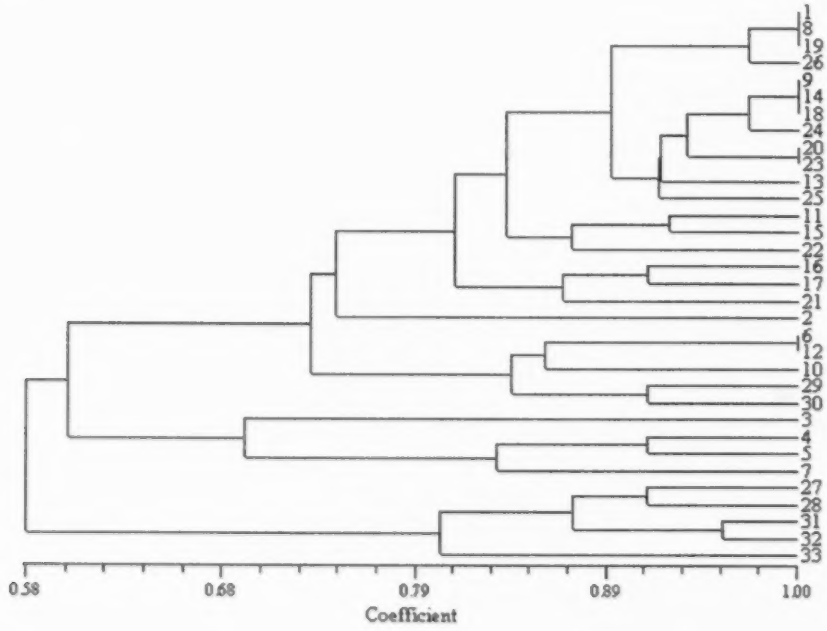

Figure 2: Dendrogram of sea buckthorn accessions as generated from AFLP analysis using the J coefficient

Genetic Diversity within Developed Cultivars: Five cultivars developed during the project for release as cultivars were: OL-6 (sample 30), EP6476 (sample 10), FR-14 (sample 31), RC-4 (sample 32) and EP6582 (sample 33). In all three dendograms, these samples (SBCs) grouped into two broad clusters. FR-14, RC-4 and EP6582 formed a

cluster with two other accessions collected from Saskatchewan. A second distinct grouping included the 2 accessions from Rafferty along with Accessions OL-6 and EP6476.

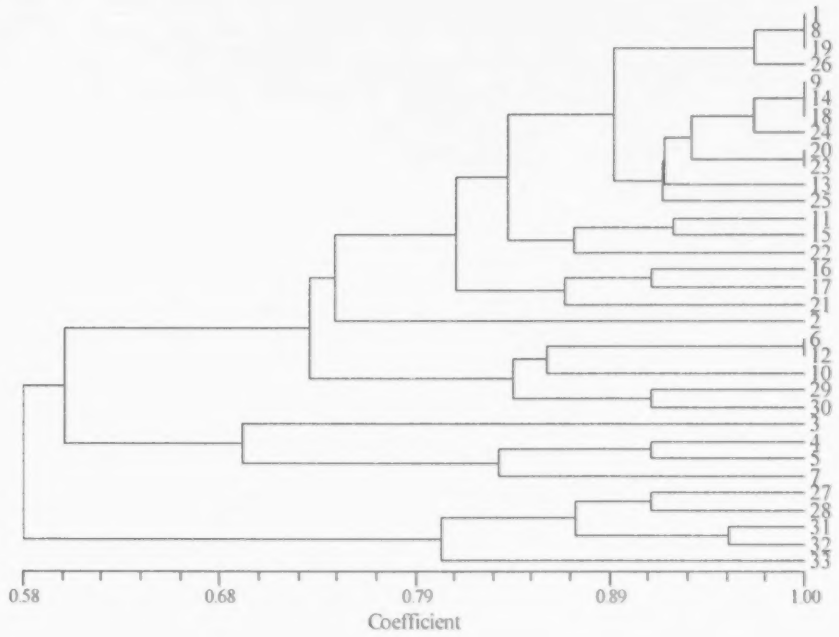

Figure 3: Dendrogram of sea buckthorn accessions as generated from AFLP analysis using the SM coefficient

The final point that could be determined from this analysis was the placement of the accession EP6469. This accession was classified as *Hippophae neurocarpa* and not *H. rhamnoides*. However, when analyzed by AFLP, this sample was found to be identical to R-41 and clustered with 4 other *H. rhamnoides* accessions. These results suggest that one of the two identical samples may have been misclassified or mislabeled. This could also indicate that the species distinction between *neurocarpa* and *rhamnoides* require additional genetic analysis.

ISSR Amplification: We obtained 24 ISSR primers (UBC Biotechnology DNA Services Laboratory) and used them to attempt to amplify satellite sequences in the sea buckthorn genome. It was hoped that this would also be used to study genetic relatedness. We were unable to establish conditions where the primers amplified DNA. However, this technique is promising and further exploration of this possibility is warranted.

Conclusion:

The AFLP technique can be used for characterizing sea buckthorn germplasm. The technique can be used to analyze sea buckthorn germplasm for assessing genetic diversity and relatedness. The results of our analysis indicate that accessions collected from Saskatchewan have as much genetic diversity as that present among accession in a random of accessions from other outside Canada sources. With possible exception of FR-14 and RC-4, there appears to be adequate genetic diversity among the remaining cultivars, which were development during the project for release. FR-14 and RC-4 appear to be genetically very close, and it may be wise to discontinue development of one these lines. The remaining accessions, while grouping into 2 clusters, are sufficiently distinct to warrant further development and release as cultivars to prairie growers. Four accessions, M6-126, N4-11, TB-2, WCS3, WCS4, are most distantly related to the cultivars were development during the project. We recommend that one or more lines derived from these accessions be considered for future cultivar development work in the province.

Finally, it must be emphasized that the above results are based on a relatively small molecular analysis. Increasing the number of primers tested, or inclusion of ISSR or RAPD polymorphisms with these AFLP results may produce differing genetic linkages than presented here.

Personnel: Ms Ravinder Grewal; Employed in January 2003

Equipment purchased: none

Impact on Saskatchewan Agriculture: The DNA fingerprinting of sea buckthorne accessions identified two accessions that are closely related, and these should be considered as one accession. The genetic analysis revealed that adequate genetic diversity exists among the cultivars developed during the project period of three years. Selection and improvement of these cultivars should continue to derive maximum genetic benefits from the cultivars developed for release.